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PROBLEMS OF SPACE BIOLOGY

VOLUME XVI

Edited by Academician V. N. Chernigovskiy

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PROBLEMS OF SPACE BIOLOGY
VOLUME XVI

Edited by V. N. Chernigovskiy

FOREWORD

Medico-biological studies related to the study and conquest of space are /3* increasing every year. This is caused by the fact that, for flights of long duration, that is, to support human life in conditions which are unlike normal conditions of man's existence and the biological objects which would have to accompany man on prolonged flights, a number of complex problems have to be solved, including a study of the general biological importance of gravitation for the vital activities of terrestrial forms of living things.

As the first step in conquering near-earth space it was sufficient to establish that spaceflight factors during short-term activity (up to two or three weeks) are not dangerous for man and other living things. The human organism, having great compensatory possibilities, adapted to flight conditions quite effectively.

However, certain physiological and other indices of vital functioning of organisms indicated that weightlessness is not completely indifferent for the organism, at least in combination with the action of other spaceflight factors. This also applies to problems of living in hermetically sealed enclosures. A large number of problems arose which demand profound theoretical research. This explains why the area of space biology and medicine has gradually become more complex and has begun to include a multitude of medical and biological problems which have to be solved before the prolonged stay of man in spacecraft can be supported.

The series of articles which are being published in "Problems of Space Biology" is sufficiently descriptive of this situation. With each volume

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*Numbers in the margin indicate pagination in the foreign text.

of this series an ever-increasing number of medical and biological problems is included in the realm of space problems.

In this collection the articles are distributed among six sections, in /4 which the problems are closely linked with sufficiently thorough theoretical research.

Studies of the action of diverse spaceflight factors on the organism of man and animals are continued, especially overloads and hyperkinesia. Several papers are devoted to study of the structure of organs and tissues under these influences.

The articles on selection of the optimum gas atmosphere for enclosed spaces are very important. A large section of the collection is devoted to problems of toxicology of an artificial atmosphere for human beings dwelling in it. This area is taking on increasing importance among space-medical problems.

For the first time, the series "Problems of Space Biology" contains papers on exobiology. They are somewhat removed from applied problems of spaceflight of the immediate future, but the results of these investigations are of undoubted interest and value. Problems of the study of the action of low temperatures and the ultraviolet portion of the spectrum may even have practical importance.

The editors will be grateful for any critical comments on this collection and on the series "Problems of Space Biology" as a whole.

Academician V. Parin

I. GENERAL PROBLEMS

/5

REACTIVITY OF AN ORGANISM IN CONDITIONS OF PROLONGED SPACEFLIGHT[1]

P. V. Vasil'yev

ABSTRACT. It was determined that weightlessness and hypodynamia lead to functional reorganization of the organs of circulation, respiration, and excretion, analyzers, regulatory systems of the organism, as well as different kinds of metabolism. These shifts inevitably lead to changes of the general reactivity of an organism, which is closely linked to its resistance. The article presents the author's own data and data from the literature which show that under the effect of weightlessness and hypodynamia the orthostatic and vestibular stability decrease, sensitivity to infections increases, resistance to accelerations and physical stress decreases, and reaction to medication changes.

The data introduced show the necessity of more thorough and comprehensive study of the problem of reactivity of an organism in conditions of prolonged space flights and possible ways of controlling it.

As is known, prolonged spaceflights will be accompanied by the action of a whole complex of factors on the human organism: during ejection of a spacecraft into orbit -- noise, vibration, acceleration, great neuro-emotional stress, and during orbital flight -- dynamic weightlessness, relative hypodynamia, varying atmosphere, abnormal day and night schedule and so on. In spite of the slight inconvenience of the effects of each factor considered separately, their complex action due to summation and accumulation will always turn out to be substantial. Therefore the problem of the effect of each factor taken separately, and especially their combination, on the functional

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[1] Paper delivered at the 3rd International Symposium on the Basic Problems of Human Life in Space, Geneva, November, 1968.

state of man calls for careful study. The least studied factor is the effect of weightlessness.

Completed orbital flights and the material obtained from modeling the state of weightlessness by the method of long-term hypodynamia in conditions of an immersion medium or by means of staying in bed show that significant functional rearrangements of a number of organs and systems occur in the human organism.

Through the works of many authors it has been determined that weightlessness and hypodynamia lead to substantial changes on the part of indices of hemodynamics, breathing, organs of excretion, function of analyzers, regulatory systems of the organism and various kinds of metabolism (Buyanov et al., 1967; Panderova et al., 1967; Parin et al., 1967; 1968; Katkovskiy, 1967; Korobova /6
and Vinichenko, 1968; Kakurin, 1968; Yuganov, 1963; Komendantov and Kopanev, 1962; Myasnikov et al., 1963; Lawton, 1962; Graveline, 1962; Muller, 1963; Neuman, 1963; Graybiel et al., 1964; Voris, 1963; Dietlein, 1964; Gauer et al., 1965; Berry, 1966, 1967, and others). It should be noted that the observed functional and biochemical disturbances are accompanied in a number of cases by substantial structural changes. The degree of both the functional and morphological changes in the investigations carried out depended on the type and individual peculiarities of the organism, the nature of the experimental model and its duration.

Naturally the rearrangements noted in the abovementioned systems must inevitably lead to changes of the overall reactivity of an organism, which is closely related to its resistance. This is supported by experiments in determination of orthostatic stability, capacity to withstand accelerations, vestibular stability, sensitivity to infection, pharmacological preparations and other factors.

A number of authors showed convincingly that in astronauts after completion of flights (Gazenko, Gyurdzhian, 1965; Buyanov et al., 1966; Berry et al., 1962; Digiovanni, 1964; Lamb, 1964, and others), and also in experiments with simulation of the state of weightlessness (Pestov, 1968; Asyamolov,

Voskresenskiy, 1968; Korobov et al., 1968; Muller et al., 1964; Thomas et al., 1964, and others) when carrying out an orthostatic test, more pronounced changes in the cardiovascular system were observed (in comparison with initial data), which in a number of cases led to orthostatic collapse.

In our tests (Vasil'yev, Kotovskaya, 1965), and also in studies by other authors (Mutler, Leverett, 1955) after 20-70 hour hypodynamia lowering of resistance to accelerations of 1.5-2 G_x on the average was detected. Under the action of accelerations having magnitudes of equal value there was a higher functional stress on the principal physiological systems of the organism among the subjects tested. In subjects tested at accelerations of 8-9 G_x the average pulse rate following hypodynamia was 170 ± 11 beats/min, while during controlled rotations of the same test subjects it was 149 ± 13 beats/min. Similar discrepancies were observed in the indices of external respiration.

In our investigations (E. V. Lopayev) we noticed a lowering of the threshold of resistance of the vestibular apparatus to linear accelerations and rolling for a long time after hypodynamia.

Establishment of the fact of a lowering of the immunological reactivity of a man subjected to prolonged hypodynamia is especially important. Kraus and Raab (1961, Lawton, 1962 and others) have shown that strict bed confinement in many cases leads to the onset of serious complications in the form of pneumonia, exacerbation of jaundice, thrombosis of veins, etc. G. P. Mikhaylovskiy et al. (1967), in experiments with 62-day hypodynamia rhinopharyngitis, catarrh of the upper respiratory tracts, and periodontitis were noted in their test subjects. In our investigations with 70-day hypodynamia we observed complications in the form of acute otitis, appendicitis, urethritis and pyodermia. Undoubtedly these complications are a consequence of the lowering of the natural resistance of an organism in its different sections. In works by M. I. Kozar' (1966) and G. P. Mikhaylovskiy et al. (1967) it has been shown that during hypodynamia the phagocytic activity of the blood is suppressed, the lysozymic activity of the saliva and the gastric juice dies out, and the

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bactericidal function of the skin is reduced. According to data of O. G. Alekseyeva (1965) even in conditions of short-term flights changes in immunological reactions are observed, which are quickly restored to normal after the flight.

Of course the onset of diseased conditions during flight, and also the necessity in certian cases of increasing the resistance of the organisms of astronauts to extreme environmental factors (ionizing radiation, hypoxia, acceleration, etc.) may demand the use of medications. As is known, during the flight of the spacecraft "Apollo 7" and "Apollo 8" the astronauts had to resort to taking medications for medical reasons.

It has been established that in prescribing medication it is extremely important to consider the reactivity of the organism because one chemical (pharmacological) substance, given in the same dosage, can in some conditions (in one functional state of the organism) bring undoubted benefit, but in other cases it may cause irreparable damage (Belay et al., 1966). In experiments on different types of animals it has been determined that after the influence of prolonged transversely directed stresses an increase of sensitivity to cardiac glucosides, narcotics and certain other medications is observed as well as a reduction in sensitivity to analeptics and analgesics; reaction of the organism to administration of mediators of the vegetative nervous system changes (Belay et al., 1964, 1965, 1967; Parin et al., 1965). For example, as can be seen in Figure 1, an increase of motor activity of white mice upon administration of amphetamine after stress was less pronounced than in the control animals, which obviously indicates a reduction of the specific effect of the preparation. In our experiments we also obtained data which indicated /8 a lowering of the analgesic effect of *promedol* after the action of transverse stresses.

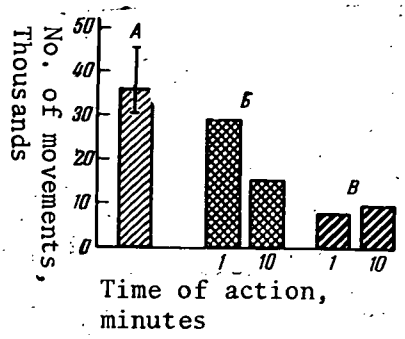

Figure 1. Number of Movements of the Animals After Intraperitoneal Injection of Phenamine in a Dose of 10 mg/kg Before the Load (A) and After its Action for 10 units (B) and 40 Units (C).

Numerous studies have determined that reaction of an organism, which is affected by penetrating radiation, to medication depends on the degree of severity of the disease, the period of its development, the dosage and the nature of the preparation (Belay et al., 1961; Vasil'yev and Saksonov, 1958, and others). In some cases there was an increase, in others - a reduction, and in others - a distortion of the reaction to administered agents.

Change in the composition of the ambient atmosphere (hypercapnia), hypoxia) also causes a change in the reactivity of an organism to pharmacological substances (Dmitriyeva, 1960; Sorinson, Postnikova, 1952, etc.). We determined that in an atmosphere with an increase of the amount of carbon dioxide (3-10%) the stimulating effect of analeptics (caffeine, Corazol) on respiration in rabbits decreases significantly or does not manifest itself at all. In similar experimental conditions the pharmacological action of phenamine decreases. This is partially demonstrated by results of a study of motor activity of white mice after administration of phenamine under normal conditions and for varying periods of adaptation of the animals to a hypercapnic atmosphere.

Data are now available from experiments on animals and studies with the participation of human subjects which indicate that sensitivity of an organism to pharmacological agents also changes after hypodynamia. L. A. Kravchuk and V. G. Ovechkin (1968), conducting experiments on white mice, established that

after 37-day hypodynamia all stages of narcosis induced by the use of barbamyl are characterized by a number of peculiarities; the stage of excitation sets in more quickly, the complete effect occurs later and the duration of sleep is shorter. On the contrary, in our experiments, conducted on white rats, hypokinesia lasting 14 and 28 days was accompanied by increase of the depth and time of *intranarkon* narcosis (V. Ye. Belay and G. D. Glod) which is evidently conditioned by the development in the brain of processes of internal retard- /9
ation, noticed by R. Granp'yer (1968) also in real flight conditions of weightlessness in monkeys. By using strychnine, caffeine and phenamine in studies with simulation weightlessness in man we noticed a change in reaction on the part of a number of principal indicators, which characterize the function of the cardiovascular system (minimal, average, final systolic and pulse pressure, pulsating and momentary volume of the heart, rate of a dilation of a pulse wave and others).

The data obtained so far do not permit one to make any specific recommendations as to the specifics of prescribing medication during prolonged spaceflights, but they show the importance of conducting investigations of these problems.

Even a brief review of the problem, it seems to us, shows that weightlessness and hypodynamia, which will be unavoidable aspects of spaceflights in the near future, casue a long series of functional, biochemical and morphological shifts in an organism and lead to a change in its overall reactivity. Unfortunately this problem has not received enough attention, especially on the part of clinicians and pharmacologists who are responsible for specific sections of medical support for prolonged spaceflights.

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CERTAIN RESULTS OF MEDICAL INVESTIGATIONS ABOARD THE "VOSKHOD-2" SPACECRAFT

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ABSTRACT. During the flight of P. I. Belyayev and A. A. Leonov in the "Voskhod-2" spacecraft it was found that reaction of the cardiovascular and respiratory systems in the pre-launch period and during the majority of the stages of the orbital flight did not differ substantially from observations in preceding flights. The frequency of heart contractions and respiration rose in the cosmonauts by only 1.5-2 times during the launching.

There was also no pathological asymmetry of the oculomotor activity or slow large-amplitude oscillations, which are characteristic of a drowsy state. The number of oculomotor reactions during the first two orbits of the flight in both astronauts was increased and reached 105-110 a minute. During the remainder of the flight the number of oculomotor reactions was in the range of 10-40 a minute. A. A. Leonov's body temperature during the launch rose on the average by 1.35°. During descent the average temperature values did not differ from those recorded in preceding orbits; however, the variability of temperature indices increased.

The food ration developed for the flight made up for energy consumption, and according to the astronauts it was entirely sufficient. Weight losses for P. I. Belyayev and A. A. Leonov after one day of flight were approximately the same as for short-term ground experiments in executing the launch program.

Analysis of the blood and urine in both cosmonauts after the flight revealed shifts characteristic of a general stress reaction: increase in the urine of adrenaline and noradrenaline, 17-hydroxy- and ketosteroids, as well as creatinine, and a marked increase of lymphocytes and leukocytes in the blood.

Clinical examination for three days after the flight showed an increase in reaction of the cardiovascular system to orthostatic tests and physical loads. Electrocardiographic examination revealed slowing down of intragastric conductivity and increase of the systolic index. EEG analysis showed reinforcement of sluggish activity. Continued examination for a month after the flight revealed no deviations in comparison with the condition before the flight.

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At 11:37 on March 18, 1965 a human being left a spacecraft and entered space for the first time. The flight was made in the "Voskhod-2" spacecraft. The commander was pilot-cosmonaut P. I. Belyeyev, the copilot was pilot-cosmonaut A. A. Leonov.

The principal tasks of the flight were: checking the design of the apparatus for ensuring the exit of man from a spacecraft; possibilities of movement and functioning of an astronaut in unsupported space; conducting medical monitoring of the cosmonaut's condition during the spacewalk and the subsequent day-long flight; monitoring the radiation conditions.

In contrast to preceding designs, the "Voskhod-2" spacecraft had an air-lock chamber and systems for ensuring transfer from a hermetic cabin into deep space. The air regeneration and conditioning system did not differ in theory from that of the "Voskhod" spacecraft (Genin, Voronin, Fomin, 1965).

Preparation of the cosmonauts, with the exception of training for moving and working in unsupported space, was the same as before prior flights (Karpov, 1966). The system of medical monitoring and methodological procedure under flight conditions were described earlier in a number of publications (Gazenko, Gyurdzhina, 1965). An addition to the system of medical monitoring is the panel of the ship commander, which shows data on the rate of heart contractions and respiration of the cosmonaut outside. This enabled a more careful management of the actions and work of the pilot while outside. Another important feature was that both pilot-cosmonauts were instructed in conducting medical tests and they successfully accomplished them in flight.

Clinical-physiological examination of the cosmonauts before and after /12
the flight was conducted according to the same pattern as in previous flights.

Studies showed that the dose of radiation during the flight and exit from the ship with calculation of the RBE factor did not exceed 0.3 rem. Such a dose could not have a harmful effect on the health of the cosmonauts (Volynkin et al., 1966). Studies of biological objects aboard the

spacecraft and in the space suit of A. A. Leonov during exit (Zhukov-Verezhnikov et al., 1966) confirm this.

Proper scheduling of eating and drinking periods had great importance for positive emotional attitude, good state of health and high degree of efficiency on the part of the cosmonauts during the flight, especially because the exit of A. A. Leonov from the spacecraft into deep space demanded additional energy expenditure. The daily food ration was accordingly set at a level of 3,250 large calories as against 2,530-2,770 large calories on the flights in the spacecraft "Vostok" and "Voskhod".

On the eve of the launch both cosmonauts had meals similar to those aboard their spacecraft. The eating schedule was the same as recommended for the flight: four times a day with intervals of 4-5 hours in daytime and 7-8 hours at night. Because the interval between morning breakfast on Earth and the first meal in flight might be as long as 8 hours, breakfast on launch day was boosted to 1,000 large calories. In addition, the cosmonauts ate chocolate bars and drank tubes of juice (about 250 large calories) after putting on their space suits 1.5 hours before take-off.

During the flight the cosmonauts had to take, along with their food, about 144 grams of protein, 115 grams of fat and 388 grams of carbohydrate. Their daily ration contained 4 milligrams of vitamins B_1, B_2, B_6, 100 micrograms of vitamin B_{12}, 4 milligrams of folinerin, 20 mg of pantothenic acid, 200 mg of vitamin C, 30 mg of vitamin PP, 100 mg of vitamin P, 2 mg of vitamin A, 50 mg of vitamin E. The assortment of food products included natural processed products, packed in plastic packages or tubes. The daily standard of water allowance was increased to 2 liters as against 1.25 liters for the crew of the "Voskhod" spacecraft. In addition, the cosmonauts could take about 878 grams of water with the juices and products.

In selecting their eating time the cosmonauts relied to a large degree on general circumstances and their appetite. According to their subjective feelings they ate no less than usual. They drank little water although P. I. Belyayev noticed increased thirst, which was connected with specific nature of the food ration. The cosmonauts experienced no sense of discomfort

in the gastrointestinal tract during the flight. At the end of the flight the food ration they had on board was estimated as sufficient by the cosmonauts.

Additional opinions regarding the effectiveness of the food ration and metabolism in the organism of the cosmonauts during the flight is provided by analysis of the indices of nitrogen metabolism. For two to three days before the flight the amount of total nitrogen in the daily urine was 11.5-13.6 grams for P. I. Belyayev and 12.4-13.8 grams for A. A. Leonov. Two days after the flight we found a somewhat greater amount of total nitrogen in the daily urine of the cosmonauts: 16.9 grams for P. I. Belyayev, 14.7 grams for A. A. Leonov. In converting the above figures for total nitrogen to protein we obtain 105.6 grams for P. I. Belyayev and 91.2 grams for A. A. Leonov after the flight. If we compare these values to the amount of protein in the food ration of the cosmonauts (about 140 grams) we conclude there was a sufficient amount of protein in the food. A slight increase of excretion of nitrogen with the urine after the flight could be explained by the extent of the activity of the cosmonauts and the neuro-emotional stress, which is in conformity with the rise of adrenalin activity and increase in the urine of adrenaline, noradrenaline, 17-oxy- and ketosteroids, and also creatinine. The creatinine factor rose after the flight from 29 to 36 for P. I. Belyeyev, and from 24 to 32 for A. A. Leonov. (Results of the biochemical stuides were obtained on the basis of data of I. S. Balakhovskiy, T. A. Orlova, et al.). /13

The body weight of the cosmonauts did not change substantially after the flight: In the two-day study P. I. Belyayev lost 1.0 kg in comparison with his pre-flight weight, and A. A. Leonov lost 0.9 kg. The cosmonauts lost approximately the same amount during the ground execution of the space capsule exit program in conditions of rarefaction of the surrounding atmosphere: P. I. Belyayev lost 0.65 kg and A. A. Leonov 0.9 kg. It should be noted that in the preceding flight on the "Voskhod" the body weight of the cosmonauts decreased in comparison with initial weight by 2.5-3.0 kg on the first day.

The specific gravity of the urine in an analysis made two days after the flight of P. I. Belyayev and A. A. Leonov was slightly increased. For A. A. Leonov we noticed a small increase in elimination of sodium and chlorine with the urine, the amount of mucus increased slightly and casts appeared.

During a hematological study before launch we noticed an increase of lymphocytes in both cosmonauts: from 30 to 36.5% in P. I. Belyayev, from 21.5 to 37.5% for A. A. Leonov. The total number of leucocytes increased to 8,400-8,800 cells per cubic millimeter [mm^3]. A similar tendency of the white blood to change was observed in other cosmonauts during examination at launch time. Such changes can be explained by the reaction of overall stress of an organism.

Post-flight study of the blood revealed a marked increase in the number of leucocytes (8,400-9,000/mm^3). The blood formula and other hematological indices were normal. The amounts of sugar, chlorine, and cholesterol in the /14
blood did not differ from pre-flight levels.

The general state of health of the cosmonauts in the pre-launch period was good, their mood was heightened, and both actively participated in flight preparation. The pulse rate in the morning was 60-64 beats/min for P. I. Belyayev; breathing rate 8-10 cycles/min, maximum arterial pressure - 100, minimum 65-70 mm Hg. For A. A. Leonov at this time the pulse rate was 52-60 beats/min, breathing rate was 10-12 cycles/min, maximum arterial pressure - 110-115, minimum - 65-70, pulse pressure was - 45-50 mm Hg (Table 1).

In a period of 5-minute readiness for flight the cosmonauts remained outwardly calm, but the rate of heart contractions increased to 83 for P. I. Belyayev and to 82 beats/min for A. A. Leonov. The breathing rates increased to 18 and 20 cycles/min, respectively. In comparison with previous cosmonauts, the changes in pulse rate and breathing were moderate. The mean square deviations and variation factors of certain EKG indices as the launch drew closer also changed comparatively little (Table 2).

TABLE 1. VALUES OF ARTERIAL PRESSURE, PULSE RATE AND RESPIRATION FOR COSMONAUTS BEFORE AND AFTER ORBITAL FLIGHT ABOARD THE "VOSKHOD-2" SPACECRAFT.

Astronaut	Index	Preflight			Postflight			
		6 Mar	14 Mar	17 Mar	18 Mar	21 Mar	22 Mar	17 Apr
P. I. Belyayev	Pulse	64	64	60	60	80	56	64
	Respiration	8	--	10	10	10	--	--
	Arterial pressure	100/65	100/70	100/65	100/70	115/80	105/70	105/70
A. A. Leonov	Pulse	56	56	52	60	72	56	60
	Respiration	12	--	10	10	12	--	--
	Arterial pressure	115/65	110/65	115/70	115/70	120/50	115/65	110/65

Note: We used data of V. G. Terent'yev, A. V. Nikitin et al. in compiling the table.

The cosmonauts easily withstood the loads during entry of the spacecraft into orbit; the changes in physiological functions were less than for most other cosmonauts in previous flights (Vasil'yev, Kotovskaya, 1965). After entry into orbit P. I. Belyayev and A. A. Leonov did not notice any unpleasant sensations or illusions. The pulse rate in the transition period from g-loads to weightlessness fluctuated from 88 to 122 per minute. Changes in /15
length of electrical systole and certain other EKG indices in this period corresponded to the rate of heart contractions and were not pronounced (Table 2).

On the first orbit of the flight the rate of heart contractions and respiration in both cosmonauts remained high: up to 105 for P. I. Belyayev, up to 100 beats/min for Leonov. The breathing rates were 21 and 24 cycles/min, respectively. A sharp increase of fluctuation of the above indices in comparison with the prelaunch period (Table 3) was characteristic of P. I. Belyayev, which is evidently connected to neuro-emotional stress, conditioned

by preparation for the first accomplishment in space of the exit of A. A. Leonov from the spacecraft.

TABLE 2. CHANGE IN CERTAIN EKG INDICES FOR P. I. BELYAYEV AND A. A. LEONOV DURING THE FIVE-MINUTE COUNTDOWN AND AT THE START OF THE PERIOD OF WEIGHTLESSNESS.

Astronaut	Index	Statistical Index	Prelaunch Period, min.		Beginning of Weightlessness, min.				
			- 5	- 1	1	2	3	4	5
P. I. Belyayev	Pulse rate	M	83	78	92.3	88.2	103.4	122.4	111.1
		σ	6.89	6.93	8.95	8.03	9.82	14.08	17.33
		C	8.3	9.0	9.7	9.1	9.5	11.5	15.6
A. A. Leonov	Pulse rate	M	82	80	103.4	88.2	90.9	90.9	109.1
		σ	7.7	8.3	8.55	8.49	6.27	7.27	8.73
		C	8.7	9.2	8.27	10.2	6.9	8.0	8.00
P. I. Belyayev	Length of ventricular complex	M	0.33	0.34	0.32	0.32	0.31	0.29	0.29
		σ	0.011	0.012	0.010	0.013	0.012	0.027	0.018
		C	3.3	3.6	3.13	4.04	3.86	9.34	6.09
A. A. Leonov	Q - T, sec.	M	0.34	0.34	0.32	0.34	0.34	0.34	0.32
		σ	0.012	0.012	0.016	0.016	0.010	0.009	0.017
		C	3.4	3.5	4.8	4.6	2.9	2.7	5.3

Note: In Tables 2 and 3, M is an average value; σ is the mean square deviation; C is the coefficient of variation.

The stage of direct entry from the spacecraft into deep space was the most stressful one for A. A. Leonov. His heart contraction rate at this time increased to 152, and during return into the air-lock chamber from space temporarily rose to 162 per minute. His breathing rate at this time reached maximum values (26-36 per minute). The changes in certain indices during entry into deep space are shown in Table 4 (3-14th min.). The data in the table show that EKG changes were somewhat abnormal. For example, in spite of the /18 increase of rate of heart contraction (reduction of R-R interval), the auriculoventricular conductivity slowed down. The length of the electrical systole (Q-T interval) had a comparative rise, while an especially pronounced

increase was observed after the return of A. A. Leonov into the airlock chamber, against a background of shortening of heart contractions. The amplitude of the R and T spikes during Leonov's spacewalk was also comparatively high, while an especially significant increase in the size of the R spike was observed after return into the airlock chamber against a background of shortening of the heart rhythm. The indicated changes are evidently related to the preceding physical and neuro-emotional stress. However, it should be noticed that these changes continued only briefly and, like the pulse rate, quickly returned to the initial values shortly after Leonov's return to the ship.

In the next period (3rd to 7th orbits) the rate of heart contraction and respiration revealed a tendency to decrease in both cosmonauts (see Table 3). The greatest reduction was noticed on the 7th orbit when the cosmonauts were resting or were carrying out simple operations. Belyayev's heart contraction rate decreased in this period to 73 and Leonov's to 54 beats/min. A similar tendency of the reactions of the cardiovascular system of the cosmonauts was noticed by other authors (Yazdovskiy et al., 1964; Bayevskiy, Gazenko, 1964; Kas'yan et al., 1964; Vasil'yev et al., 1965; Kas'yan et al., 1965; Balakhovskiy et al., 1966). The breathing rate during this period for each cosmonaut was 14 cycles/min on the average. Fluctuation of the heart contraction rate in P. I. Belyayev decreased, but no similar reduction was noticed in A. A. Leonov. Fluctuation of the breathing rate for both cosmonauts in comparison with the initial period of the flight did not change substantially.

In the second half of the flight (13-18th orbits) the heart contraction rate rose noticeably. The increase was especially pronounced before descent of the spacecraft. On the 17 and 18th orbits Belyayev's heart contraction rate was 94-111, and Leonov's was 75-86 beats/min. Respiration frequency changed significantly less in the second half of the flight in comparison with the first (see Table 3).

Electrocardiogram indices in the first and second halves of the flight corresponded basically to changes in pulse rate.

TABLE 3. RATE OF HEART CONTRACTIONS AND RESPIRATION OF THE COSMONAUTS IN THE PRELAUNCH PERIOD AND IN ORBITAL FLIGHT.

Astronauts	Statistical Index	Prelaunch Period (5 min. countdown)	Orbital Flight (Orbits)												
			1st	2nd	3rd	4th	5th	6th	7th	13th	14th	15th	16th	17th	18th
Heart contraction rate, beats/min.															
P. I. Belyayev	M	83	105	90	84	76	84	79	73	77	87	97	90	94	111
	σ	6.89	16.2	10.2	8.48	8.51	7.56	7.9	6.57	6.93	6.96	12.6	11.7	11.3	10
	C	8.3	15.4	11.3	10.1	11.2	9.0	10	9.0	9.0	8.0	13.0	13.0	12.0	9.0
A. A. Leonov	M	82	100	118	60	64	70	70	54	70	77	71	73	75	86
	σ	7.7	8.2	5.9	5.8	8.6	6.5	7.0	5.3	9.9	10.8	9.3	9.8	8.4	8.6
	C	8.7	8.2	5.0	9.6	13.4	9.3	10	9.8	14.1	14.0	13.1	13.4	11.2	10.0
Respiration rate, cycles/min.															
P. I. Belyayev	M	17.7	21	22	18	18.5	18.6	17.6	14.0	18.8	18.8	18.2	18.5	15.8	76.6
	σ	4.42	7.35	7.48	7.74	6.68	6.51	6.69	5.18	7.52	7.90	8.55	7.40	6.16	6.18
	C	25	35	34	43	36	35	38	37	40	42	47	40	39	35
	M	20	24	22	18	17	18	19	14	18	20	18	19	18	20
	σ	6.70	6.91	8.14	3.76	5.78	6.30	4.75	4.62	4.32	6.60	4.32	5.32	5.94	5.40
	C	33.5	28.8	37	32	34	35	25	33	24	33	24	28	33	27

TABLE 4. CERTAIN EKG INDICES OF COSMONAUT A. A. LEONOV IN THE PRELAUNCH PERIOD AND DURING EXIT FROM THE SPACECRAFT INTO DEEP SPACE (SECOND ORBIT OF THE FLIGHT).

Indices	Pre-launch period	1st *	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th	19th
Auriculo-ventricular conductivity (P-Q), sec	0,14	0,15	0,16	0,15	0,16	0,16	0,20	0,20	0,17	0,18	—	0,17	0,18	—	—	0,18	0,18	0,17	0,18	—
Length of ventricular complex (Q-T), sec	0,34	0,38	0,37	0,36	0,34	0,33	0,32	0,32	0,31	0,31	0,32	0,33	0,33	0,32	0,33	0,40	0,41	0,41	0,42	0,41
Length of R-R, interval, seconds	0,69	0,56	0,45	0,45	0,41	0,39	0,39	0,40	0,39	0,39	0,39	0,39	0,40	0,37	0,38	0,44	0,51	0,56	0,66	0,59
Systolic index, %	49,3	68,0	82,3	80,0	83,0	84,5	82,0	80,0	79,5	79,5	82,0	84,5	82,5	86,5	86,8	91,0	80,5	73,0	63,5	69,5
Height of EKG spikes (in relative units), mm																				
R	9,9	10,2	9,8	8,3	9,9	9,8	10,5	10,0	8,6	9,2	—	8,6	8,4	—	—	12,5	12,3	12,0	12,5	—
T	3,4	2,7	4,1	4,7	3,6	3,8	4,7	4,3	3,8	4,4	—	—	4,7	—	—	4,9	4,3	3,3	4,0	—

*Minutes

Translator's Note: Commas indicate decimal points.

In the first half of the mission, the length of the electrical ventricular systole, in parallel with the tendency towards reduction of pulse rate, rose from 0.30-0.34 to 0.35-0.42 second, while the systolic index decreased from 57-53 to 43, or 3.8%. The auriculo-ventricular conductivity in the initial /19
period increased from 0.14-0.16 to 0.17-0.18 second. In the second half of the flight, along with an increasing pulse rate, the length of the electrical ventricular systole decreased from 0.40-0.35 to 0.36-0.30 second, while the systolic index rose from 38-43 to 52-55% (Table 5). EKG changes, with the exception of a few abnormal temporary indices for Leonov in the exit period and single nodal extrasystoles for Belyayev in the same period, as well as during preparation for an descent of the spacecraft to Earth, were observed within the limits of the physiological fluctuations and did not differ from those observed on previous flights.

The pulse in both cosmonauts was rhythmic and strong in a medical examination on the first day after landing: 58 beats/min for Belyayev and 52-54 for Leonov. Breathing was quiet, with a rate of 12-14 cycles/min. Lowering of pulse and breathing rate was accompanied by symptoms of moderate general fatigue. Both cosmonauts, on their own initiative, did not sleep during the flight, wanting to conduct as many observations as possible. They stayed awake even after landing until meeting with doctors.

In a clinical examination on the third day after landing, the pulse rate increased from 60-64 to 74-80 per minute. During a standing-up test the rate was still higher. Whereas the pulse rate before the flight increased to 76 per minute for both cosmonauts, after the flight it increased to 102 for Belyayev, and to 96 for Leonov. Systolic and diastolic arterial pressure under conditions of relative quiet increased by 15 mm Hg for Belyayev. For Leonov the diastolic pressure fell by 15 mm Hg.

Electrocardiographic study after the flight showed an increase in the time of intragastric conductivity during shortening of the heart cycle. The QRS interval increased in Belyayev from 0.08 to 0.09 second, and from 0.09-0.10 to 0.11-0.12 second for Lenov. The sum of the heights of the P spikes with the three standard leads increased respectively by 55 and 69%,

the sum of the spike heights T decreased approximately by 10%, and the sum of the heights of the R spikes did not change. The systolic index exceeded the proper values by 11 and 7%. Vector analysis of the electrocardiograms did not reveal any appreciable dynamics of the value and direction of the QRS and T vectors and the intragastric gradient "G".

Ballistocardiographic study also did not reveal any peculiarities.

Hemodynamic indices for Leonov were normal. In comparison with background data, a reduction of cardiac output from 100 to 84 ml was noticed along with an increase of pulse rate (+ 15%). Minute circulation, ratio of the moduli of elasticity and other indices did not change substantially.

Under flight conditions the cosmonauts conducted a one-time study of /21
certain indices of external respiration. The vital capacity of the lungs of the cosmonauts decreased somewhat: from 4,600 to 3,500 ml for Belyayev and from 5,200 to 4,200 ml for Leonov. On the third day after landing the vital capacity of the lungs was within normal limits, although somewhat less than initially: 4,400 for Belyayev, 4,900 ml for Leonov. During the flight the respiratory volume and pulmonary ventilation for both cosmonauts approximately doubled. After the flight, in comparison with the prelaunch values, an increase of pulmonary ventilation was noticed (for Belyayev by 51% and 18% for Leonov). The daily energy expenditures, according to gas exchange data, exceeded the initial data on the third day after landing by 28% for Belyayev and 20% for Leonov.

Study of body temperature during the flight was undertaken only for Leonov. The limits of its fluctuations and average values are given in Table 6. In analyzing the data in the table it can be seen that during the exit of Leonov into deep space the average level of body temperature in comparison with the first orbit increased by 1.35°; simultaneously the minimum and maximum temperature values rose, and were at their maximum in comparison with the data recorded on the other orbits. A sharp body temperature increase on the second orbit was related to the great physical effort and neuroemotional stress demanded of Leonov in executing the exit program. During simulation of the flight program in a tank for testing temperature- and

pressure-measuring equipment a body temperature increase was also noticed for Leonov, but it was less (by 0.8°C on the average).

On the fourth orbit Leonov's body temperature was lower than on the first orbit (35.2° instead of 36.0°). Lowering of the temperature evidently reflected the general condition of the cosmonaut and not an increase of body surface ventilation. A similar lowering of body temperature can be seen on the seventh and fifteenth orbits, when the cosmonaut was in a state of relative quiet and ventilation was normal. /22

On the 16th and 17th orbits, i.e., at the end of the flight, the average values of body temperature (36.0-36.2°) were the same as on a number of other orbits; minimum temperature values (35.0-35.4°) corresponded to those recorded in rest periods on the 1st, 4th, 7th and 15th orbits, but the maximum values differed substantially from those recorded on other orbits and approached those observed during physical and neuroemotional stress during the space-walk. At the end of the flight a sharp increase in the range of body temperature fluctuations for Leonov was noticed, which was evidently conditioned by the preparation for the first execution of manual control of systems for landing the spacecraft on Earth.

After the landing, the body temperature for both cosmonauts was within normal limits and did not differ substantially from the temperature recorded in the prelaunch period.

Studies in flight of pain and tactile sensitivity as well as two-dimensional spacial and stereognostic sensation showed these indices did not change substantially under conditons of weightlessness for either cosmonaut. Only the recognition of certain numbers written on a paper gave any difficulty. Belyayev was not able to correctly identify the number 5, and Leonov the number 3 (the size of the digits was 2 × 1.5 cm).

Analysis of the dynamics of oculomotor activity of the cosmonauts during flight showed that on the first two orbits the number of EKG waves reached 105-110 per minute for both cosmonauts. On the third and fourth orbits the number of oculomotor reactions decreased and subsequently fluctuated

within comparatively narrow limits: 10-40 movements per minute. At the end of the flight the number of eye movements increased somewhat. Such dynamics of the electrooculogram are in agreement with the heart contraction rate and the breathing rate, and reflect the general state of the cosmonauts. Similar changes of electrooculograms were observed in previous flights (Akulinichev et al., 1965).

In analyzing the height and shape of EOG curves it could be seen that throughout the flight the cosmonauts' eye movements were quite symmetric. Pathological asymmetry, characteristic of disturbance of the eye muscle tone, was not recorded. Sluggish and large-amplitude oculomotor reactions, which are characteristic of the period of falling asleep or sudden fatigue, were not detected.

Low and average-size movements predominated in the EOG in flight during the increase of oculomotor activity. Group oculomotor activity, connected to observation of quickly-passing phenomena, in most cases lasted 3-7 seconds. /23
At times a minor optokinetic nystagmus with frequency of 3-4 movements per second lasting 1-1.5 seconds was observed against the background of (or after) increased oculomotor activity.

During an electroencephalographic examination of the cosmonauts on the third day after the flight we noticed in Belyayev a predominance of beta rhythm with a frequency of 18-20 Hz and amplitude of 35 μV, single and brief series of slow waves with a frequency of 5-6 Hz pronounced in the right central-occipital section; individual sharp waves with a frequency of 30 Hz and amplitude of 30 μV. In the case of Leonov, the EEG on the third day after flight showed a regular alpha rhythm with a frequency of 18-20 Hz and amplitude of 10-15 μV and single, slow, low-amplitude waves with a frequency of 5-6 Hz in the left frontal-central lead. In comparison with background studies we noticed an increase of slow electrical rhythm for both cosmonauts, which evidently supports the increase of inhibitor processes in the cortex of the brain in relation to general fatigue phenomena.

TABLE 5. CERTAIN INDICES OF CARDIAC ACTIVITY OF COSMONAUTS IN THE PRE-LAUNCH PERIOD AND UNDER ORBITAL FLIGHT CONDITIONS (AVERAGE DATA).

Astronaut	Index	Orbit of flight													
		Pre-launch period	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th
P. I. Belyayev	Auriculo-ventricular	0,15	0,16	0,17	0,17	0,17	0,17	0,17	0,17	0,17	0,17	0,16	0,16	0,16	0,14
A. A. Leonov	conductivity (P-Q), sec	0,14	0,14	0,16	0,17	0,16	0,18	0,18	0,15	0,15	0,15	0,14	0,15	0,14	0,14
P. I. Belyayev	Length of ventricular	0,33	0,30	0,32	0,34	0,35	0,34	0,34	0,35	0,35	0,33	0,32	0,32	0,32	0,30
A. A. Leonov	complex (Q-T), sec	0,34	0,34	0,36	0,42	0,40	0,38	0,38	0,42	0,40	0,38	0,39	0,38	0,38	0,36
P. I. Belyayev	Length of R-R interval	0,72	0,57	0,66	0,71	0,79	0,71	0,76	0,82	0,78	0,69	0,62	0,67	0,64	0,55
A. A. Leonov	sec	0,69	0,60	0,51	1,00	0,94	0,86	0,86	1,12	0,85	0,78	0,84	0,82	0,80	0,70
P. I. Belyayev	Systolic index, %	46	52,6	49	49	44,3	48	45	43	45,2	48,0	51,6	48	50	54,5
A. A. Leonov		49,3	57	50,5	42	42,5	44	44,3	37,5	47,4	48,8	46,4	46,3	47,5	51,4
P. I. Belyayev	Height of R spike	9,91	9,33	10,2	11,8	10,4	10,0	9,9	10	8,5	10	9,7	11,2	—	—
A. A. Leonov	(in relative units)	5,7	4,3	3,9	3,9	4,2	4,0	4,2	4,1	5,1	4,9	4,0	4,4	3,1	2,7
P. I. Belyayev	Height of T spike	1,09	1,0	1,37	1,20	1,40	1,30	1,30	1,30	1,40	1,90	1,20	1,30	0,84	1,40
A. A. Leonov	(in relative units)	3,4	3,2	2,8	4,9	4,0	3,6	4,0	4,0	3,2	3,4	3,0	3,6	3,8	3,6

Translator's Note: Commas indicate decimal points.

TABLE 6. BODY TEMPERATURE OF A. A. LEONOV DURING VARIOUS ORBITS OF THE ORBITAL FLIGHT, °C.

/21

Orbits	Average	Min	Max.	Orbits	Avg.	Min.	Max.
1st	36,00	35,5	36,8	13-й	36,00	36,0	36,1
2nd	37,35	36,6	37,6	14-й	36,30	36,3	36,4
3rd	35,20	35,1	35,4	15-й	35,50	35,0	35,8
4th	36,50	36,3	36,6	16-й	36,00	35,4	37,4
5th	36,30	36,0	36,6	17-й	36,20	35,0	37,6
6th	35,45	35,4	35,5				
7th							

Translator's Note: Commas indicate decimal points.

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STUDY OF FEATURES OF HIGH-INTENSITY NOISE EFFECTS DURING SPACEFLIGHT

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ABSTRACT: Experiments were performed to study the effect on man of high-intensity noises whose frequency range, length and volume corresponded to the conditions of the active period of spaceflight. The effect of 125-126 and 114-116 db of noise on the auditory and motor analyzers, and also on the condition of the pulse and blood pressure, of 24 subjects was studied in 105 experiments during exposure for 20 minutes. It was found that noise at 125-126 db during the given exposure time causes unfavorable reactions on the part of the above-mentioned indices. The authors conclude that there is no danger from noise at 114-116 db for 20 minutes, considering the demands and particular features of spaceflight.

The problem of the effect of noise on man in conditions of spaceflight is still far from solution. Characteristics of noises during various segments of a flight (active portion, orbital phase) differ in exposure time, intensity and spectral composition. During the active part of the flight, cabin penetration by high-intensity noise is characteristic; in the orbital phase, acoustic stimuli of average intensity act on the cosmonaut. Studies of the effect of noises of average intensity on man show the possibility of preservation of high auditory efficiency with efficient normalization (Krylov, 1965; Yuganov, Krylov, Kuznetsov, 1966; Krylov, 1967a; Krylov, 1967b; Yakovleva, Mantsev, 1967; Kuznetsov, 1968). /25

In the literature, there are only a few mentions of calculated noise levels in the active part (Jacobs, 1960; Konecci, 1959; Lott, 1964) and special studies on correspondingly permissible noise levels are lacking. To study features of the action of high-intensity noise on the human organism and determine their permissible values relative to the active stage of flight, we conducted special experiments. In solving this problem we figured that recommendations for permissible noise levels in the cabin must be worked out with

consideration of the specific conditions of cosmonaut activity in the active and orbital parts of the flight. In connection with the fact that during the active flight the cosmonaut receives radio signals and carries out commands related to them, there was a necessary limitation of the noise level to values which would not cause substantial changes in the reception of sound signals.

We conducted studies on 24 subjects with a 20-minute exposure to noise at 125-126 db (Series I) and 114-116 db (Series II).

We performed 105 tests in all.

We first determined hearing thresholds for air conductivity in the range of frequencies from 125 to 10,000 Hz by the method of automatic audiometry, and also differential thresholds for intensity above 1,000 Hz. The total effect of noise on the human organism was evaluated by the changes of pulse rate, value of systolic and diastolic arterial pressure, as well as the time of responsive motor reaction to light stimulus before and after the action. We assumed that the latter methodology is the most important for evaluating the effect of noise on the quality of reception and execution of commands by a cosmonaut. Intensity and noise exposure time in Series I were determined with the help of the calculated data of Jacobs (1960). /26

It was determined that the effect of wideband noise at 125-126 db causes a rise of the hearing threshold in the test subjects, an increase in time of simple response reaction and change in the indices of blood pressure. The thresholds of hearing rose by 12-15 db after exposure, causing complaints of head pain, noise, ringing and feeling of stuffiness in the ears for 20-40 min. after the experiment. Differential thresholds for intensity, as a rule, decreased and returned to original levels only after one to two hours. Such an effect could be interpreted as a result of reversible parabiotic changes in the hair cells of the organ of Corti (Ravdonik, 1950; Vinnikov, Titova, 1961; Temkin, 1962). The pulse rate after the noise decreased as a rule; according to data of T. A. Orlova (1965), E. V. Lapayev and I. Ya. Borshchevskiy (1966), this confirms the pronounced overall action of noise. The blood pressure indices increased mostly due to the increase of diastolic pressure. The pulse pressure decreased in most subjects.

	Extreme fluctuations (relative to background)
Blood pressure, mm Hg	
Maximum	+10 -5
Minimum	+15 -5
Pulse	-5 -10
Pulse, beats/min.	-4 -10

The time of response reaction to light stimulus also increased. With average starting data before the experiments of 0.24 second ($\sigma \pm 0.14$) it reached 0.35 second ($\sigma \pm 0.13$) by the end of the action with a reliability factor of 3.4.

The results confirm that noise levels of 125-126 db lead to functional changes in a number of organs and systems, which characterize the pronounced overall unfavorable action of noise on the human organism (Vozhzhov and Sapov, 1960; Arkad'yevskiy, 1962; Orlovskaya, 1963). This forced us to continue our search for permissible noise levels.

We found that 110 db noise lasting up to 30 minutes was satisfactorily endured by the subjects (Borshchevskiy, Lapayev, 1966). For this reason, in /27
the experiments of the second series, the subjects were exposed to noise for 20 minutes at 114-116 db. It turned out that in this case the changes in auditory functions were insignificant. The thresholds of hearing increased overall by 5.0-7.5 db; differential thresholds based on intensity changed in various ways.

Changes in differential thresholds based on intensity (in db) during action of 114-116 db noise can be shown as follows:

Before action (A)	0.9	1.3	0.9	1.0	1.2
After action (B)	0.8	1.5	0.9	0.9	1.3
B - A	-0.1	+0.2	0	-0.1	+0.1

The same picture was evident in the dynamics of the indices of arterial pressure during the action of 114-116 db noise:

Blood pressure mm Hg	Extreme fluctuation (relative to background)	
Maximum	+10	-10
Minimum	+12	-8
Pulse	+4	-8

The time of simple motor reaction to photostimulation increased as a rule. But restoration of this index to the original level occurred by the 10th to 15th minute after the end of the action.

Comparative evaluation of the results of Series I and II shows a rather marked distinction of physiological effects during the action of 125-126 and 114-116 db noise. The dynamics of pulse rate, value of arterial pressure, time of response motor reaction and indices of auditory function during the action of 125-126 db noise confirms that this noise level cannot be considered permissible. However, 114-116 db noise causes substantially smaller changes in the function of corresponding organs and systems. These changes have, as a rule, a diverse nature and may be evaluated as a result of individual reactions to noise.

Considering the singularity and short acting time of a noise stimulus, and also the insignificant effect of the physiological changes on the organism during the action of 114-116 db noise, we suggest that the indicated cabin noise level is permissible during the active part of the flight. Such conclusions are based on data from experiments on the action of noise on the unprotected ear. The use of earplugs naturally allows adjustment of the recommended limits. Modern earplugs lower external noise at low frequencies by 15-20 db (Gromov, Kuznetsov, 1968). Undoubtedly this circumstance should also be considered in /28 calculations of permissible cabin noise levels aboard a spacecraft during the active part of the flight.

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II. PHYSIOLOGICAL FUNCTIONS OF AN ORGANISM DURING EXTREME ACTIONS

EFFECT OF IMMERSION ON CERTAIN MOTOR FUNCTION INDICES

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ABSTRACT: The article presents results of a study of certain indices of a simple movement with visual adjustment and without it, and also a determination of the peculiarities of the functional state of the segmentary apparatus of the spinal column during the period of organization of voluntary movements of man during prolonged stay in a physiological solution.

Reduction of the threshold value of the H-reflex during a three to four day stay in conditions of immersion is seen as a consequence of the increase of reflector excitability of alpha-motor neurons of the spinal column.

Increase of the average time of the latent period in each of the test subjects on the second day of their stay in conditions of an immersion medium gives the basis for supposing that in these conditions the functional state of the supraspinal centers is lowered.

Comparative evaluation of execution of a motor problem with visual adjustment and without it shows the well-defined nature of shifts during shut-off of the visual adjustment both in background examinations and after immersion on all days of recovery. The immersion medium has a different effect on the postural tonic and phase musculature, and correspondingly also on the nature of execution of motor acts.

Study of the effect of the immersion medium on the state of the motor /29
apparatus of man in relation to development of space travel has not only an applied but also a general theoretical importance for special physiology.

Change of gravitational influences in ground conditions is created during flights in airplanes, fast elevators and special machines. In these cases short-term conditions of weightlessness arise.

Conditions of weightlessness are accompanied by a whole set of changes which include quite different functional systems of an organism. As a rule changes of the adjusting and postural-tonic reflexes are observed. The influence of brief periods of weightlessness causes the appearance of

compensatory mechanisms and quick restoration of coordination of the elementary motor acts (Kas'yan, Kopanev, Yuganov, 1964; Chkhaidze, 1965; Yuganov et al., 1962; Gerathewohl, 1954). A study of astronauts' handwriting shows how it improves during repetition of the test in flight conditions (Gazenko, 1964). With the cosmonauts V. Komarov and B. Yegorov we noticed that the time of executing a motor task in the beginning of the flight increased appreciably, and then became shorter during repetitions.

The most adequate ground model of weightlessness must not only create the physical effect of this state but also reproduce the psychological and physiological influence.

Models which came close to reproducing the action of reduced gravitation might be immersion media.

During immersion in water the muscle system experiences an effect which closely resembles conditions of weightlessness. However, the ability to endure water immersion is limited by time. Nevertheless, the high compensatory capacity of the muscle system which is observed in conditions of weightlessness during spaceflights, during prolonged hypokinesia and immersion in water lets us suppose that at the bottom of the changes there is one and the same mechanism, which ensures reliability of execution of motor tasks in the listed conditions. /30

In tests of a seven-day stay in water (Graveline, 1962) with exposure times of 6, 12 and 18 hours, the most detailed study was made of the cardiovascular system, and less thorough studies were made of the mechanisms of changes of motor function (Beckman et al., 1961; Graybiel, Clark, 1961; Howard et al., 1967; Vogt, 1965).

During the action of the water immersion for varying periods of time we observed atrophy of the skeletal musculature, especially of the postural-tonic musculature, in separate instances, deterioration of the Rhomberg attitude, and lowering of muscle strength. Noticeable changes in coordination of the upper and lower extremities were not detected. Precision tasks were executed with slight deviations (Graybiel et al., 1961; Beckman et al., 1961; Graveline, 1962; Vogt, 1965).

These data prove the high compensatory capacity of the human motor system.

Undoubtedly, longer action of the natural conditions of weightlessness could cause disorder of these mechanisms of compensation. In prolonged flights (up to 1,000 days, as planned in the USA) we must necessarily provide for prevention of irreversible muscular disorders.

The literature on the effect of changed gravitational field on the state of the human motor function does not give a complete idea of the nature of these changes. Evidently the varying extent of the changes observed by us is determined by the length of the stay in water, by the level of coordination possibilities of man, and also by methodological possibilities of the procedures used in the study.

In relation to the fact that weakening of gravitational forces might variously affect the postural-tonic and phase muscle groups, we studied certain indices of simple movement with visual adjustment and without it, and we also determined the peculiarities of the functional state of the segmentary apparatus of the spinal column during organization of the voluntary movement of a man during prolonged stay in a physiological solution. Five healthy men from 22 to 30 years old with good level of physical preparedness participated in the experiment.

The experiment was carried out in a tank 251 cm × 113 cm × 167 cm. Water temperature of 34° ± 0.5°C was maintained constantly. In order to ensure /31
constancy of electrolyte balance, sodium chloride was added to the water, 9 grams of NaCl per liter of water. Thus, the test subjects were in a physiological solution. The position of the test subject on a net ensured fixation of the body and enabled complete muscle relaxation, the depth of immersion was 0.5-0.3 meters, and the head was kept above water.

The test subjects stayed in the immersion fluid for five days (120 hours). For two days before immersion we examined the physical (on a special dynamographic foot lever) and postural-tonic muscles. An electromyogram (EMG) of *m. gastrocnemius* and *m. longissimus dorsi* and a dynamogram (DG) were recorded. From the oscillograms we determined the following parameters of simple movement:

latent time of electrical activity (LTEA), latent time of movement (LTM), time of movement (TM), length of electrical activity (LEA), amplitude of electrical activity (AEA), frequency of electrical activity (FEA), percent of deviation from the given precision of movement (% of error).

The general procedure for carrying out the method is as follows. Upon a visual signal, the test subject must make a quick precise movement (extension of muscles of the spine and underside bending of the shin joint). Recording of the dynamogram is achieved by the aid of a special unit which consists of a support, a movable lever goniometer. One end of the goniometer is attached to the support, the other end to the spine of the test subject in the area of the shoulder blade by the aid of a special suction disk.

A similar method was used for evaluating the precise force of the phase muscles. With this aim the test subject, sitting in an armchair, placed his right leg on a foot lever, which is rigidly connected to a potentiometer. At that moment electromyograms of the lateral head of the gastrocnemius muscle were recorded and the angle of the shin joint changed, whose value was translated into absolute units of muscle force.

Extension of the postural-tonic muscle and the underside flexion of the phasic muscles were executed ten times, at first with visual adjustment, and then repeated as many times without visual control.

After a five-day stay in the immersion medium the test subjects underwent a three-day examination for evaluating the degree of action of the test conditions, and also for determining the nature of recovery. In this case the movements were executed at first without visual control, then with visual control and again without it.

The functional state of the segmentary apparatus of the spinal column during organization of voluntary movement was evaluated by monosynaptic testing. Recording of the reflector response (H-reflex) was achieved by means of surface /32
electrodes with the gastrocnemius, soleus, and anterior tibial muscles on the three-channel "Diza" electromyograph. For stimulation of the nerve in the popliteal depression, a rectangular pulse with a length of 1 msec from the "Mul'tistim" electric stimulator through a transformer outlet was used.

The control value of the H-reflex was 10-20% of the maximum. Its stability was checked in ten consecutive tests before and after the experiment, and also after each 20-30 executed movements. Underside flexing of the shin joint, which was executed in response to a visual signal, served as a model of voluntary movement during study of motor pretuning[2].

The latent period of this motor reaction was tested by the H-reflex after each 10 milliseconds on ten occasions.

In Table 1 we present data of the background study which shows that in underside flexion of the shin joint without visual control, the LTM and LTEA decrease when the force developed during the movement increases. Evidently, the increase of the TM, LEA, AEA and FEA are related to the latter.

TABLE 1. DYNAMICS OF VARIOUS PARAMETERS OF VOLUNTARY MOVEMENT (FLEXION OF SHIN JOINT) BEFORE AND AFTER IMMERSION.

Parameters	Before immersion		After immersion								
			First day			Second day			Third day		
	*	**	**	*	**	**	*	**	**	*	**
LTEA, msec	290	243	157	276	196	175	277	227	229	204	228
LTM, msec	330	290	204	331	259	221	311	223	280	287	292
Amplification error, %	16	28	4	16	28	8	12	20	36	20	28
AEA, KV	1195	1327	1440	1141	1225	1312	1024	1336	1070	1026	1031
FEA, Hz	92	102	100	108	112	102	94	108	102	86	98
LEA, msec	452	471	354	526	496	447	483	512	450	412	459
TM, msec	597	695	545	595	574	691	734	746	499	500	471

* Visual control

** Without visual control.

[2]By the term pretuning, we mean the functional changes that occur in the segmental apparatus of the spinal cord prior to the execution of a movement.

From Table 2 it can be seen that during voluntary movement (flexion of the spine) in which primarily the postural muscles participate, precision in executing movement with visual control and without it changes insignificantly. /33
According to electromyographic and dynamographic indices there is contraction of LTEA and LTM, and also a change in the length of the movement itself during execution of movement without visual control in the postural muscles as well as in the phase muscles.

TABLE 2. DYNAMICS OF VARIOUS PARAMETERS OF VOLUNTARY MOVEMENT (FLEXION OF SPINE) BEFORE AND AFTER IMMERSION.

Parameters	Before immersion		After immersion								
			First day			Second day			Third day		
	*	**	**	*	**	**	*	**	**	*	**
LTEA, msec	188	175	196	173	180	196	204	173	167	183	178
LTM, msec	313	283	313	307	266	268	275	267	259	289	267
Amplification error, %	13	13	14	13	14	10	12	10	13	11	13
AEA, μV	813	810	655	604	658	760	655	596	723	663	768
FEA, Hz	179	173	167	154	161	167	148	146	154	145	156
LEA, msec	1154	1058	1137	1091	1088	1045	1091	1076	1161	1041	1059
TM, msec	1129	1068	1119	1044	1069	1080	1053	1090	1185	1033	1041

* Visual control
** Without visual control.

The threshold value for provoking the maximum H-reflex of the gastroncnemius muscles in background studies was 20-24 volts based on nominal values of the electric stimulator. The LTEA during underside flection in the shin joint was 150-190 milliseconds.

Statistical analysis shows that the latent period of the motor reaction can be divided into three phases according to the nature of the changes of the value of the H-reflex of the gastrocnemius muscle. The first phase consists of the interval of time from supplying of a visual signal up to 60 milliseconds before movement; series II — from the 60th to the 30-20th milliseconds,

and series III — from the 30-20th millisecond up to the beginning of the front of the EMG. Figure 1 illustrates the percentile increase of the amplitude of the H-reflex before movement. The initial value of the H-reflex at rest is taken as 100%. Point M proves that during phase I of the latent period the value of H-reflex does not vary. In the time intervals from 60th to 40th millisecond the amplitude of the H-reflex increases smoothly, then after the 40th millisecond a sharp increase of amplitude is noticed.

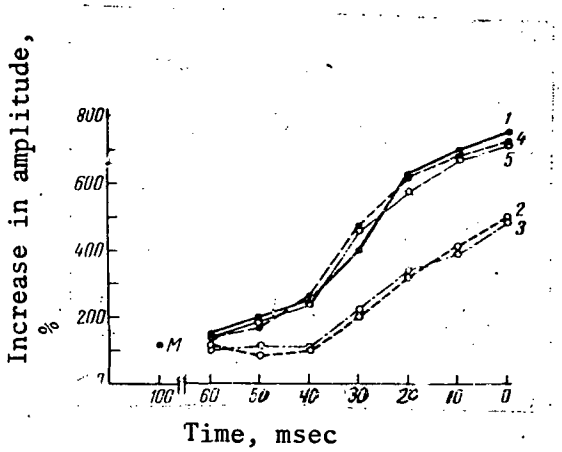

Figure 1. Graphic Dependence of Percentile Relationship of Amplitude of H-reflex and Time of Application of Electrical Stimulus to Nerve Prior to Movement. M, initial value of H-reflex at rest; 1, Before immersion. During period of immersion: 2-3rd day; 3-4th day. After immersion: 4 — after 3-4 hours. 5— on second day.

In conditions of immersion a gradual increase of LTEA is observed which reaches 240-270 msec by the fourth /34 day. At this point the threshold for causing the maximum H-reflex the other way is lowered. For two days it is equal to 12-14, on the third — 8-10, and on the fourth day — 6-8 volts.

A statistical analysis shows that during location of the test subjects in the immersion medium substantial changes occur in the characteristics of the motor pretuning. For two to three days dispersion of the amplitude of the H-reflex increases in all testable time intervals, while in phases II and III of the latent period this increase is greater than in phase I (Table 3).

On the third and fourth days disorders in the motor pretuning become more pronounced. The characteristic growth in amplitude of the H-reflex which is proper for phase II of the latent period, begins to drop off, i.e., readjustment of the segmentary apparatus of the spinal column which is related to the future voluntary movement occurs only in phase III when it is lacking in phase II (2, 3, 4 on Figure 2; 4, 5 on Figure 1).

TABLE 3. COEFFICIENTS OF VARIATION OF AMPLITUDE OF H-REFLEX OF GASTROCNEMIUS MUSCLE PRIOR TO MOVEMENT, BEFORE, DURING AND AFTER IMMERSION OF SUBJECTS.

Measurement periods	Time, sec.								
	At rest	100	60	50	40	30	20	10	0
Background	11,3	12,4	18,4	20,4	17,63	18,4	19,6	19,53	12,4
Immersion									
Third day	13,0	14,1	46,6	38,8	43,8	40,6	29,8	31,2	16,4
Fourth day	13,3	16,46	58,6	53,7	60,7	48,4	37,82	34,3	16,6
After immersion									
After 3-4 hrs.	14,0	12,2	54,75	38,75	45,7	49,53	30,29	31,4	15,66
Second day	15,3	15,6	32,8	35,9	34,6	39,7	27,3	21,1	14,75
Third day	15,0	16,0	24,6	29,4	30,4	34,8	24,2	17,6	14,4

Translator's Note: Commas indicate decimal points.

Two more facts come to one's attention: in conditions of immersion absolute excitability of the segmentary apparatus of the spinal column increases (lowering of the threshold for causing maximum H-reflex), while the relative excitability of the motor neuron pool before movement, on the other hand, decreases (see Figure 2). The time of accumulation of amplitude of the H-reflex is strongly linked to the start of the electromyogram and is not related to the overall time of the latent period. Independently of whether the mean value of the latent period changes or remains constant for each of the test subjects, the first authentic increase of the H-reflex is observed 60 milliseconds before the movement.

In comparing the results of the characteristics of execution of movement /35
(underside flexion in the shin joint) without visual control before and on the first day after water immersion it is possible to notice a reduction of LTEA, LTM and the force developed during the movement, and also the length of the movement itself (see Table 1).

Comparison of these data with results of execution of the movement with visual control indicates that five-day immersion substantially affects the characteristics of precision movement. However, the rate and amplitude of the electromyogram increase insignificantly.

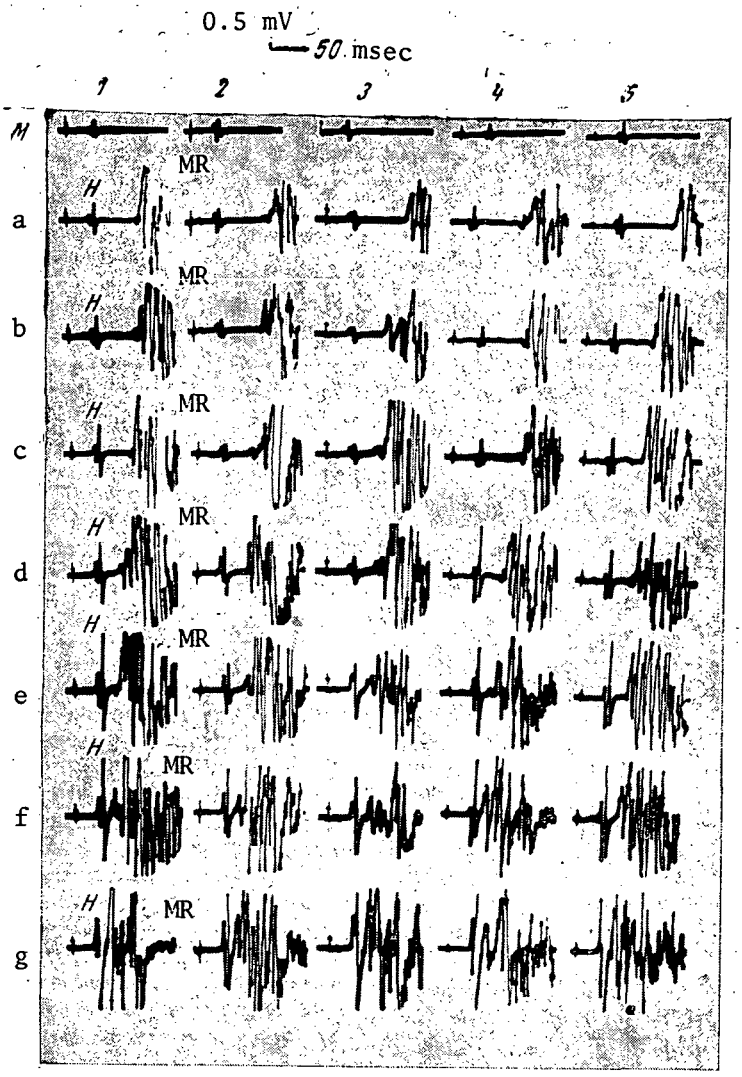

Figure 2. Change in H-Reflex of Gastrocnemius Muscle Before Voluntary Movement Prior to, During and After End of Immersion. Time, (In msec) prior to appearance of first EMG pulse; a, 60; b, 50; c, 40; d, 30; e, 20; f, 10; g, 0; H, H-reflex; MR, motor reaction. Other symbols are the same as in Figure 1.

Analysis of the study on the first day after the experiment shows that the repeated execution of movements without visual control is accompanied by loss of the capacity to reproduce a given precise movement.

Such a phenomenon continues to be preserved on the second day of the recovery period. By the third day the observed disorders are expressed by an increase of LTM ıLTEA. The TM is shortened, and the LEA becomes greater.

Comparison of the results of the characteristics of execution of a voluntary movement (straightening of the spine) without visual control before and after immersion (see Table 2) shows that on the first day after the experiment the precision of execution of movement practically does not change, but only an insignificant increase of LTEA and LTM is observed. Remaining indices decrease slightly. Tasks executed with visual control and repeatedly without it on this same day exhibit certain changes in comparison with the background data. For instance the TM and LTEA increase somewhat, LTM decreases. Change of the remaining data is insignificant.

On the second day of recovery, precision of execution of movement improves appreciably, error without visual control is 10%. Change of remaining indices fluctuate within insignificant limits. Visual adjustment and its /37
absence did not affect the relative constancy of the parameters. The third day is characterized by a decrease of LTM and LTEA, percent of error without visual control is higher. Remaining indices change little.

Three to four hours after five-day immersion the threshold for causing maximum H-reflex remained reduced and was equal to 12-16 volts, but after twenty-seven to twenty-nine hours (second day of recovery) it was near the starting values. By this time recovery of even the latent period of the motor reaction is observed.

It can be seen from Figure 1 that 3-4 hours after immersion in water substantial changes occur in the motor pretuning in comparison with four-day stay of test subjects in the immersion medium.

On the second day of the recovery period the principal disorders in the motor pretuning disappeared with the exception of dispersion of the H-reflex, which continued at an increased level in phases II and III of the latent period.

On the third day the nature of the motor pretuning was similar to the original state.

The postural-tonic and physical musculature, like the entire muscle system, which governs human movement under normal conditions, is subject to significant weakening by submersion in an immersion medium. There is a decrease in the afferent and efferent impulsation, stretching of the joint and tendon apparatus, an increase in body length — all of which affect the degree of change of joint angles.

When a human being is immersed in physiological solution, the impulsation from the mechanoreceptors drops sharply. The water pressure is distributed uniformly over the entire body, and the muscular forces directed at maintenance of posture, which usually counteract the force of gravity, are reduced to practically zero.

These factors govern the change of the indices of the dynamic and bioelectrical properties of the postural-tonic and phasic muscles, as well as the functional state of the segmentary apparatus of the spinal cord. The more pronounced significance of the dynamic changes in the phasic muscles may be associated with an increase in the general irritability of the segmentary apparatus of the spinal cord, which is retained following a long stay by the subjects in the immersion medium, regardless of the initial, natural motor activity.

A disruption of the work of the phasic muscles is expressed by a change in the muscular forces and bioelectric activity during performance of a given movement. This level of the forces during phasic movements increased much more after immersion for 2 or 3 days. The strength of the postural-tonic muscles during extension was altered very slightly under the influence of the experiment.

Any muscular activity in excess of the minimum requirements for performance of standard tasks must serve as a measure of excess effort. The higher standard of evaluation of motion is characterized by an optimum level of applied forces. /38

The possible reasons for changes in the activity of the postural-tonic and phasic muscles are governed by the state of their viscoelastic properties, tonus and the state of the connective apparatus. Undoubtedly, the observed facts are linked to some degree with muscular atrophy.

The obtained results on changes in the segmentary apparatus in organization of the voluntary movements confirm the significant effect of the immersion medium. One of the early consequences of this effect is the increase in excitability of alpha-motor neurons.

It has been experimentally shown that the post-radical deafferentation of one or of several segments of the spinal column increases the excitability of motor neurons to the reflector and supraspinal effects and also to the activating and inhibiting action of chemical agents (Veber, 1962; Kennon, Rozenblyut, 1951; Kostyuk and Savos'kina, 1959; Morison et al., 1941; Teasdall and Stavraki, 1953 et al.).

In the work of Ya. M. Kots (1968) it was shown that in man the partial ischemic deafferentation of the shin muscles exceeds the reflector excitability of the spinal motor neurons.

Reduction of the threshold value for evoking the H-reflex on the third to fourth days of immersion conditions and also lowering of the critical level of the amplitude of the H-reflex by 300% before movement confirms the increase of excitability of the segmentary apparatus of the spinal column to the reflector and supraspinal effects. The observed facts agree with modern concepts.

Lack of a characteristic growth in amplitude of the H-reflex in phase II of the latent period of the motor reaction on the third-fourth day of stay of the test subject in immersion conditions may be related to weakening of the mechanisms of regulation of posture. However, this problem calls for further study.

Increase of the average time of the latent period in each of the test subjects on the second day of confinement in immersion medium conditions gives a basis for supposing that in these conditions the functional state of the cortical centers is lowered.

It is known that the time of the spinal component of the motor reaction is a constant value (60-55 milliseconds) and does not depend on the overall time of the latent period (Mart'yanov, 1968). Evidently, the increase of the

latent period in our case is due to a lag in the higher level of the central nervous system. The works of Moruzzi and Magoun (1949) show that the /39
recticular formation has a diffuse tonic effect on the cortex. The activity of the reticular formation is supported by pulses which advance along the collaterals of the specific sensory tracts, the bases of which are tracts which conduct kinesthesic afferentation.

In interpreting the results, one could assume that limiting the motor activity in immersion conditions decreases the onset of proprioceptive impulsation in the reticular formation, which leads to reduction of its activity and, as a consequence of this, to reduction of the tonus of the cortex. A change of the latent period is probably also related to this.

Comparative evaluation of execution of a motor task with visual adjustment and without it shows in large part the unique nature of shifts during inclusion of visual adjustment both during background examinations and after immersion on all days of recovery.

Execution of a motor task by the postural-tonic musculature and the phase muscles is characterized by certain peculiarities.. Consequently, immersion has a varying effect on these muscle groups.

It should be noted that in spite of the change of the indices studied, a given movement is executed. Ability for execution of a motor task with visual adjustment and without it on a background of changed motor pretuning, and also of dynamic and bioelectrical indices confirms the high conpensatory capabilities in the work of nerve structures, which are responsible for accomplishing movements. In addition, one of the indices of compensatory mechanism is the rapid recovery of the functional state of the segmentary apparatus, noticed in the first three to four hours after immersion.

Hopf et al., (1967) showed experimentally that rapid voluntary movements are mainly organized centrally and may be executed without noticeable effect of additional regulating. Regulation of conditions, which is disturbed by the effect of immersion, may take place even in natural weightlessness conditions.

The experimental data presented permit one to determine the effect of immersion on the nature of movements which are accomplished by the postural-tonic and phase groups of muscles, and also on the processes of organization of voluntary movements. The results confirm that the central nervous system has a number of mechanisms which ensure a broad range of compensation for execution of motor tasks.

Accumulated experimental material, obtained during investigation of hypodynamia of various duration, of immersion and of actual flights in spacecraft does not give a complete picture of the mechanisms of compensation of /40
the motor function of man. Study of these problems is of the utmost necessity for refining methods of selection and training of the crew.

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BASAL METABOLISM UNDER CONDITIONS OF SIMULATED WEIGHTLESSNESS

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ABSTRACT: The effect of twenty-four hours' water immersion on the level of basal metabolism and certain functions of external respiration of man was studied.

Three series of tests were conducted.

In the first series, the test subjects were in a water medium. In the second series, they remained for twenty--four hours in bed. The third series was the control. In the first and second series the food ration was the same.

Under conditions of water immersion the indices of basal metabolism of the consumption of oxygen, of elimination of carbon dioxide, pulmonary ventilation and the coefficient of the use of oxygen are higher than under conditions of staying in bed. This confirms that such models of weightlessness, such as hypodynamia (bed conditions) and water immersion lead to different levels of metabolism.

The study of the level of exchange processes in man at rest and under /41
physical stress in conditions of weightlessness are necessary for design of life support systems of a spacecraft, and also for developing an energetically balanced standard of feeding cosmonauts.

In the literature there are data which are principally devoted to problems of functional space of the cardiovascular system under conditions of hypokinesia.

It was established that hypokinesia of varying duration causes manifestation of tachycardia, a change in EKG, a lowering of the tone of arterial and venous vessels, a lowering of the pulsation and minute volumes of the blood (Buyanov, 1964; Buyanov et al., 1966; Vanyushina, 1963; Georgiyevskiy and Mikhaylov, 1968; Kakurin and Tokarev, 1964; Katkovskiy, 1967; Matyushina and Korolev, 1964, Graveline, 1962).

Thus, during hypokinesia there occurs a retuning of the functional state of the cardiovascular system. This permits one to suppose that the level of

the metabolic processes and respiration also undergo changes. This is supported by work (Vanyushina et al., 1966; Katkovskiy, 1967; Lawtong, 1962; Pierson, Rich, 1965), which indicates a slowing of metabolic processes during hypokinesia. However, the authors conducted investigations under conditions of hypodynamia (bed conditions) and in an attitude of "average physiological rest."

Only the work of Howard et al., (1967) contains a report on dynamics of a change in external respiration and gas exchange for a six-hour immersion at rest. In this work the authors emphasized a certain increase in rate of respiration, pulmonary ventilation and use of oxygen.

In our work we have attempted to explain the orientation of possible shifts in the level of metabolism under conditions of twenty-four hour hypodynamia.

For this purpose, we conducted three series of tests, each of which used eight very healthy young men.

In the first series, basal metabolism in the test subjects was studied under conditions of fluid immersion. The experiment was conducted in a bath with a capacity of 2 m^3 at a water temperature of 34-36°. The test subject, in a / 42 horizontal position on a special bed for rigid positioning, was submerged in water up to the collarbone. During the experiment, the subject ate at a specific time. At night he was permitted to sleep.

In the second series, the basal metabolism was studied while the test subject was in bed for twenty-four hours. The order of eating and sleeping was the same as in the first series.

Because there is a clear relationship between basal metabolism and total caloric value of food, which reflects the state of metabolism under conditions of rest (Graveline, 1962), the test subjects in the first and second series were placed on a special ration, near in composition to balanced nutrition (1:1:4).

In the literature there are data which confirm the necessity of placing the test subject on a specific ration for several days before conducting tests of basal metabolism (Slonim and Makarov, 1949). Such a transition in the two series was done four days before the beginning of the test.

The caloric content of the ration was 2,600-2,700 kcal/day and was maintained up to the end of the experiment with actual feedings of 2,300-2,700 kcal.

The third series of the experiment was the control. A study of basal metabolism and function of external respiration in this series was conducted for evaluating the general conditions and the composition of the test subjects. The basic difference of the control series from the two others consists in that the time in bed did not exceed 10-12 hours, and in place of a balanced ration, a normal daily diet was given. Deviation of the actual basal metabolism from what should have been expected in this series did not exceed 5%, which confirms the normal state of the test subjects. The basic indices of external respiration corresponded to the expected values.

For studying basal metabolism and certain functions of external respiration samples of exhaled air were collected from the test subjects of each series at the same time by the same methods. The exhaled air was collected in Douglas bags for five minutes through s valved chamber with the nose clamped shut. Analysis of the collected air was conducted on an Orrs-Fisher gas analyzer with control tests on a Haldane apparatus.

Ventilation of the lungs was determined with a water gasometer.

The volumetric indices of respiration and gas exchange were brought to normal conditions (BTPS and STPD).

The data obtained were subjected to statistical analysis.

Analysis of the results of the study of basal metabolism and external respiration showed a main trend to shifts of metabolic processes under conditions of water immersion.

In comparing the indices of the first and second series of the experiment it was noticed that basal metabolism, respiratory frequency (RF), minute volume of respiration (MVR), oxygen consumption, carbon dioxide excretion, respiratory coefficient (RC), coefficient of oxygen consumption (OC), which characterized /43
the effectiveness of pulmonary ventilation, did not change in the same way in individual test subjects.

However, in the tested subjects of the first series the average indices of basal metabolism and functions of external respiration were somewhat higher than in the second series (Table).

TABLE. AVERAGE INDICES OF BASAL METABOLISM AND EXTERNAL RESPIRATION (M ± m).

Indices	Series of Experiments		
	First	Second	Third
Basal metabolism, kcal/ 24-hour period	1764 ± 99	1663 ± 99	1770 ± 85
Breathing rate, min	11.8 ± 0.81	10.7 ± 1.30	10.2 ± 0.88
Respiratory volume, ml	580 ± 89	565 ± 1.07	561 ± 92
Minute volume of respiration, liters	6.50 ± 0.75	5.84 ± 0.52	5.73 ± 0.25
Oxygen consumption, ml/min	254 ± 10.9	240 ± 13.0	255 ± 11.3
O_2 excretion, ml/min	204 ± 18.3	193 ± 15.4	200 ± 12.1
Respiratory coefficent	0.79 ± 0.032	0.8 ± 0.021	0.77 ± 0.018
Coefficient O_2 utilization, ml/liter	44.2 ± 1.7	42.4 ± 2.2	----

Thus, under conditions of immersion pulmonary ventilation increased by 12.1% RF increased by 10.3%, RV by 2.6%, level of basal metabolism increased by 6.1%, oxygen consumption increased by 4.8%, excretion of carbon dioxide correspondingly by 5.7%, OU (oxygen utilization) increased by 4.24% and RC changed insignificantly.

The level of basal metabolism, oxygen utilization and excretion of carbon dioxide in test subjects in the first and third series of the experiments were practically constant. However, the MVR in the subjects of the first series of the experiment was higher than the third by 13.4%, the RF by 11.5% and the RV increased by 3.3%.

When we compared the indices of basal metabolism and external respiration in the subjects of the second and third series, we detected changes in other functions of external respiration than in the first and third series. In this

case the MVR, RF and RV indices showed practically no differences.

However, basal metabolism in the second series was lower than in the third series by 6.4%, the use of oxygen decreased by 6.3%, and elimination of carbon dioxide fell by 3.6%.

Basal metabolism in all the series was practically identical. If the indices of basal metabolism and external respiration in the test subjects of the /44
third series are taken as the initial ones, the test subjects of the second series will show a reduction of basal metabolism, oxygen utilization and excretion of carbon dioxide (see table).

The level of reduction of basal metabolism and oxygen utilization coincide with similar data in the work of Katkovskiy (1967) derived in an experiment with hypokinesia for twenty days (first to third days).

Indices of pulmonary ventilation, frequency and depth of breathing in test subjects of the second series were practically the same as those in the third series.

In the first series of studies, in comparison with the second and third, we noticed a significant increase of MVR, RF and a significant increase of RV. However, Beckman et al. (1961) mention lowering of respiratory volume to 80% in the period of immersion in comparison with control data, and attribute this to water pressure. Nevertheless, in the work of Howard (1967) data are reported which confirm the periodicity of change of RV in relation to immersion time. Thus, for example, the author notes that after two hours of immersion the RV increased by 4.9%. After four hours it fell by 7.3% and after six hours it again increased by 11.3% in comparison with the initial level. Consequently, the average value of RV is somewhat higher than the original level. Results of our tests of RV agree with these data. From all of the indices of external repsiration in all three series respiratory volume changed least of all. More pronounced changes were seen in the MVR. Its increase is explained by a slight increase of RV and RF.

Water immersion without compensated or balanced breathing creates conditions of negative intrathoracic pressure, increasing the transmural pressure and

leading to a greater increase of the flow of blood volume within the chest (Lazaris and Serebrovskaya, 1963; Seryatin, 1962; Howard et al., 1967). It is possible to suppose that these conditions are capable of causing a slight increase in the surface of the ventilated alveoli. In addition, the excess (superatmospheric) pressure on the chest (14.7 mm Hg on the average) during inspiration creates a certain additional load on the respiratory musculature. These conditions provide a basis for supposing that breathing in conditions of immersion is carries out at a higher energetic level. It is possible also to suppose, however, that the organism as a whole (and especially the respiratory system) adjusts physiologically in a period of twenty-four hours and changes over to a more economical (optimal) functioning cycle which shows up in changes in the RV, RF and OC. Nevertheless, this new steady-state level of functioning of the respiratory system under conditions of water immersion is somewhat higher than the initial and agrees with the data of Bulenkov et al. (1968) and Howard (1967). /45

Basal metabolism at the end of one day of bed rest was the same amount below that at the end of twenty-four hours of immersion in the control (third) series as well. This reduction of basal metabolism in comparison with the control agrees with data in the work of Katkovskiy (1967) and Deitrick et al. (1948).

Twenty-four hour immersion did not have a substantial effect on the change of respiratory coefficient.

On the basis of the results obtained it is possible to conclude that twenty--four hour immersion causes a slight increase in the level of metabolism, but bed rest for twenty-four hours and prolonged hypodynamia cause its reduction.

Regarding the effect of temperature factor under conditions of water immersion, we can say that it was maintained at a optimum level, individual test subjects still lost weight. Weight losses for twenty-four hours of immersion reached 10-12%. Evidently, this is determined by metabolism. However, for broader interpretation and conclusions regarding the effect of the temperature factor on physiological functions of the human organism under conditions of immersion, further study of this problem is required.

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CHANGE IN THE CAPACITY OF MAN TO WITHSTAND TRANSVERSE STRESSES AFTER HYPODYNAMIA OF VARYING DURATION[3]

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ABSTRACT. This work presents study of the capacity of man to withstand transverse stresses (+Gx) after hypodynamia placed in a supine position for a length of 3 to 6 hours. Gradual reduction in the resistance to the action of maximum stresses was detected at time periods of hypodynamia from 7 to 15-20 hours. Later resistance to stresses was preserved approximately at the same level up to the 60th hour of hypodynamia. Similar shifts were obtained in a study of the reactivity of the cardio-vascular and breathing systems to stresses determined according to the level of pulse strain and increase of pulmonary ventilation before and after hypodynamia of varying duration. The results obtained give a basis for supposing the existence in the process of hypodynamia of two phases in reactions of an organism to stress. In the opinion of the authors, the presence of the second phase (stabilization) may support the development of a unique adaptation to conditions of hypodynamia.

Spaceflights have opened up a number of problems, one of which is the /46
study of the ability of man to withstand stresses during landing after prolonged periods of weightlessness. Studies directed toward explanation of this very complex problem have an important scientific and practical interest. Indeed, when carrying out a program of space studies it is necessary to examine thoroughly what effect on man a prolonged period of weightlessness may have. Predictions must obviously be based both on a study of flight tests and on data of laboratory experiments in which the corresponding effects of weightlessness are reproduced.

Orbital flights of cosmonauts have shown that man can stay in a state of weightlessness up to 14 days. However, studies conducted simultaneously showed certain unfavorable shifts on the part of a number of organs and systems

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of the organism of cosmonauts which point to a reduction of functional resistance of the human organism after orbital flight (Siskayan, 1964; Gazenko, 1964; Lamb et al., 1964; Berry, 1966; Taylor et al., 1966). After a flight lasting more than a day, phenomena of dehydration, demineralization and certain other disturbances were observed (Siskayan et al., 1964; Berry, 1966).

In laboratory experiments many researchers noticed similar shifts in the functional state of different systems of the organism (Kakurin et al., 1966; V. Vanyushina, 1963; Deirick et al., 1948; Graveline, McCally, 1962; Miller et al., 1964, etc.)

Of special interest are data on the effect of prolonged hypodynamia or /47
immersion in a fluid on the capacity of man to withstand stresses. Worsening of the ability to withstand the actions of longitudinal ($+G_z$) stresses, is equal to that of orthostatic probes, mentioned by practically all researchers who have worked in this field (Kakurin et al., 1963, 1966; Graveline, Barnard, 1961; David, 1961, and others).

In simulation experiments we conducted studies on the ability of man to withstand stresses ($+G_x$) with maximum periods of hypodynamia up to 20 days (Kotovskaya et al., 1964, 1967) and 28 days (Miller, Leverett, 1965). The ability to withstand stresses was not studied for longer periods of hypodynamia. In addition, tracing the change of resistance to the action of stress during varying lengths of hypodynamia was of significant interest. We therefore felt justified in setting up new experiments with varying exposure times for test subjects under conditions of hypodynamia.

We assume that the solution of these tasks will permit us to obtain data for a more complete prediction of the state of a cosmonaut during the final stage of a flight upon return depending on its varying duration.

In this work we present studies which we have at our disposal which are devoted to the problem of the ability of man to withstand transverse stresses after hypodynamia under conditions of strict test schedules lasting from three to sixty hours. Experiments on hypodynamia were conducted by L. I. Kakurin, V. I. Slesarev and other researchers.

The experiments were conducted with the participation of twenty healthy persons who had undergone special clinical examination. Evaluation of their ability to withstand stress was carried out according to a limited value of stress, acting in the "chest-back" direction ($+G_x$), at angles of 80 and 65°. The basic criterion of capacity to withstand stress at an angle of 80° was the phenomenon of relative bradycardia, less often of visual and respiratory disorders, and during the action of stresses at an angle of 65°, mainly visual disturbances.

The data obtained on the ability of man to withstand stresses and physiological reactions was statistically analyzed by the method of differences using linear unilateral regression. In addition, indices of physiological reaction of the test subjects were compiled during the action of stresses similar in value according to their absolute significance and also with consideration of the initial data. Results of the study of the ability to withstand stresses after hypodynamia are presented in Figure 1.

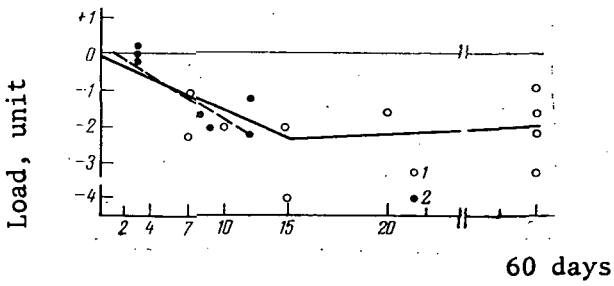

Figure 1. Change in the Ability to Withstand Transverse Stresses in Relation to Duration of Hypodynamia. 1, Individual cases with a difference of maximally variable amounts of stresses while reclining in an armchair at an angle of 10°; 2, The same, but at an angle of 25° to the horizontal.

As can be seen from Figure 1, the ability to withstand stresses after three days in bed does not change in comparison with the initial level. The gradual reduction of the resistance to stresses was observed after hypodynamia lasting seven days. After staying in bed for 15 to 20 days this reduction on the average was 2.4 units. After hypodynamia for two months the ability to withstand stress remained approximately at the same level as after fifteen-twenty days of hypodynamia (-2.2 units). Consequently, after fifteen to twenty days bedrest, further reduction of resistance

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did not develop, ability to withstand stresses stabilized approximately at the same level (-2.2; -2.4 units). Stabilization of the level of tolerance after 15-60 days of hypodynamia is confirmed statistically (coefficient of regression 0.008 ± 0.018). Restoration of the initial resistance in all the test subjects began 17 to 50 days after the two-month hypodynamia.

Similar data were obtained in a study of the reaction of the cardiovascular and repsiratory systems to stress. In Table 1 we present the rate of heart contraction in test subjects in the initial state before return, as well as under stress before and after hypodynamia of varying duration.

TABLE 1. HEART CONTRACTION RATE DURING THE ACTION OF STRESSES BEFORE AND AFTER HYPODYNAMIA (IN BEATS/MIN).

Period of investigation	Starting data		Following hypodynamia with the duration days		
	General mean, (66 persons)	Partial mean, (14 persons)	7-12	15-20	60
For 5 min. before action	76 ± 1	76 ± 3	84 ± 10	88 ± 8	91 ± 6*
For 1 min. before action	85 ± 2	86 ± 3	90 ± 15	102 ± 11	105 ± 8*
During action of a load of 8 units	124 ± 2	125 ± 4	147 ± 14	158 ± 9**	158 ± 11*
During action of a load of 10 units	128 ± 2	132 ± 5	150 ± 24	162 ± 8**	164 ± 8**

Note: The table lists mean values, mean error and level of significance in comparison to starting data. *, < 0.05; **, < 0.01. 2, In centrifuge studies the angle of inclination of the back of the armchair in all cases was 80° to the vector of acceleration.

In order to determine the authenticity of the observed changes, the shift of the frequency of caridac contractions in the subjects (14 individuals) in the original state and under the effect of 8 and 10-unit overloads during background tests prior to hypodynamia (partial mean) was compared with the rhythm of heart beats of a large group of people (66 persons), who did not participate in the experiment (general mean). It turned out that the reaction of heart activity in the test subjects of the test groups in the experiments

on the centrifuge before hypodynamia was the same as in the test subjects who did not participate in the experiment.

As can be seen from Table 1, after adhering to strict bed rest in the initial stage before the influence of stresses we noticed a pronounced increase in the frequency of the rhythm of heart activity while the frequency increased /49 along with lengthening of the time period for hypodynamia. It could be supposed that neuro-emotional tension which caused the indicated shift of heart activity in connection with previous rotation in the centrifuge, increased along with an increase in the duration of hypodynamia. During the action of stresses after 15-20 and 60 days of hypodynamia the frequency of heart beats differed significantly from the starting values. Increases of the given index after 7-12 days of bed rest turned out to be insignificant.

Dynamics of changes in the reactions of the cardiovascular system were also evaluated according to the increase of heart contraction rate during the action of stresses with a value of 8 and 10 units (Figure 2). During the first seven-twelve days of hypodynamia we observed a gradual increase in the reactivity of the cardiovascular system to stress. After 15-20 days the increase in pulse rate was +31 ± 16 beats/min on the average. One should note that after 60 days of hypodynamia the average increase in heart contraction rate turned out to be the same as after 15-20 days of bed rest (+31 ± 16). It is interesting to note that, according to the data of American scientists (Miller, Leverett, 1965) after four weeks of hypodynamia the pulse frequency under a stress of 10.6 units was also very close to the value we found (+33 ± ± 20 beats/min). Consequently, differences in increase of pulse rate after hypodynamia lasting 15-20, 28 and 60 days were practically nonexistent. It can be assumed that after 15-20 days of bed rest a period od definite stabilization of reactions of the cardiovascular system to the action of stress sets in.

Reactivity of the repsiratory system was evaluated based on the value of /50 the oxygen debt (OD) and the increase in pulmonary ventilation (IPV) after completion of rotation.

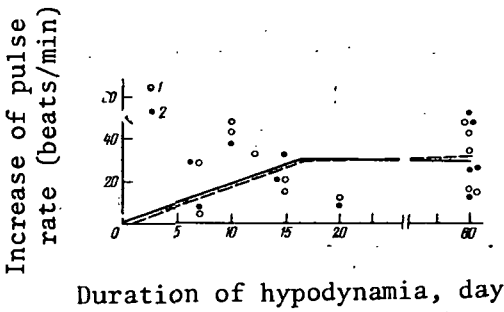

Figure 2. Change of Heart Contraction Rate During the Action of Stresses of Eight Units (1) and Ten Units (2) After Hypodynamia of Varying Duration. The angle of inclination of the back of the chair is 80° from the vector of acceleration.

The increase of pulmonary ventilation, expressed in liters, was calculated for the entire period of aftereffects of the stress as was done for determination of the oxygen debts. Changes of the oxygen debt and increase of pulmonary ventilation after hypodynamia of varying duration are presented in Figure 3.

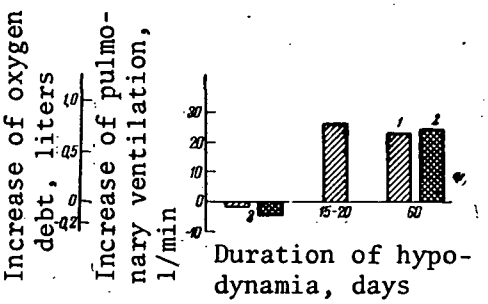

Figure 3. Shifts of Oxygen Debt (2) and Increase of Pulmonary Ventilation (1) in the Period of Aftereffect of Stresses in Relation to Duration of Hypodynamia.

From these data it can be seen that after three days of hypodynamia there were no substantial shifts in changes of indices of external respiration in comparison with background rotation. After 15-20 and 60-day bed rest the difference in change of pulmonary ventilation and oxygen debt in comparison with the initial effect was significant. Thus, the additional increase of pulmonary ventilation in the period of after-effect of stresses before hypodynamia was 17.3 liters, after 15-20 days of hypodynamia it was 43.8 liters (that is, the difference of increases was on the average 26.5 liters), and after 60 days of hypodynamia the difference in increases was 23.3 liters.

Hence, the extent of reaction of the respiratory system, according to data on increase of pulmonary ventilation, after 15-20 and 60 days of bed rest turned out to be approximately the same. The average values of the maximally

achieved stresses before and after 15-20 and 60 days of hypodynamia were similar (on the average, the difference was 0.8 unit).

Our studies were carried out with the participation of a limited number of /51
test subjects (ten persons). Therefore, in order to determine the validity of the changes observed, the data of the pulmonary ventilation and oxygen debt which were obtained for the test subjects of the test group were compared with the data of the control group of test subjects (33 persons), who did not participate in tests with hypodynamia. Conditions for the action of stresses were identical.

We made a comparison of the changes attained for each test subject separately with the proper values for the increase of pulmonary ventilation and oxygen debt corresponding to the transposed value of stresses.

The proper value of OD or IPV was calculated in each separate instance respectively for the value of the maximally achieved stress, resulting from the average data which were obtained in the control group. Later, we determined the shift of the actual value of OD or IPV in relation to the proper one. The average data obtained are presented in Table 2.

As can be seen from the data presented in Table 2 shifts of the oxygen debt and increase of pulmonary ventilation after 15-20 and 60 days of confinement /52
appeared accurate in comparison with general means. In this connection, no reliable change or increase in pulmonary ventilation was observed after 15-20 days of hypodynamia in comparison with 60 days. The difference in increases of this index after the indicated periods of hypodynamia was very slight.

Hence, a long stay of man in a horizontal position while limiting his mobility led to a reduction in the ability to withstand transverse stresses. Attainment of various levels of overload was accompanied by a significantly higher functional stress on the physiological systems of the organism. Changes of resistance to stresses after different periods of hypodynamia were varied.

For instance, after three days of hypodynamia the resistance of man to stresses showed practically no change with respect to the initial vlaues. Along with the increase in periods of hypodynamia, there was a reduction in the ability

to withstand stresses. After 15-20 days bed rest it was 2.4 units on the average. After 60 days of hypodynamia this reduction was practically the same as after 15-20 days of hypodynamia (2.2 units). The reactivity of the physiological systems during the action of stresses after the indicated periods of hypodynamia was also approximately indentical.

TABLE 2. CHANGES OF OXYGEN DEBT AND INCREASE OF PULMONARY VENTILATION IN THE PERIOD OF AFTEREFFECTS OF STRESSES BEFORE AND AFTER HYPODYNAMIA.

Index	Oxygen debt, ml	Increase of pulmonary ventilation, l
Before hypodynamia:		
General mean	21 ± 80	3.8 ± 3.3
Partial mean	+85 ± 107	+0.4 ± 3.3
After three days of hypodynamia	- 6 ± 15	-0.2 ± 1.8
Before hypodynamia:		
General mean	—	32 ± 3.2
Partial mean	—	-13.2 ± 5.9*
For 15-20 days of hypodynamia:		
General mean	—	25.9 ± 2.7
Partial mean	—	20.9 ± 6.6**
Before hypodynamia:		
General mean	788 ± 76	25.9 ± 2.7
Partial mean	-144 ± 68	-3.6 ± 3.9
After 60 days of hypodynamia:		
General mean	698 ± 78	21.4 ± 9
Partial mean	+682 ± 246**	+23.2 ± 9.4*

Notes: 1, The general mean was determined in the control group (330 persons) corresponding to the average values of stresses which were reached in the test group. 2, In the partial mean we have introduced deviations of actual values of the indicated indices from the proper values, which were obtained with consideration of the stress acting in each specific case. 3, Accuracy of the shifts was determined in comparison with the general mean (*,more than 0.95; **, more than 0.99).

Consequently, the material obtained confirmed the fact that during hypodynamia lasting 15-20 and 60 days there was no authentic relationship between the degree of reduction of ability to withstand stress, reactivity of the organism and duration of bed rest.

We suggest that the data obtained confirm the development of a process of unique adaptation to conditions of prolonged (up to two months) hypodynamia, which begins approximately on the 15th to 20th day of strict bed rest. The experimental facts which support this point of view were obtained in tests by G. I. Kozyrevskaya, L. I. Kakurin and others (1967). The authors determined that in the course of two months of hypodynamia, there arose during the second half a gradual normalization on the part of a number of indices of the water--mineral metabolism, blood system and other systems of the organism. For example, the maximum elimination of electrolytes (K, Na, Ca, F) was observed during the third to fourth week of hypodynamia; then this loss decreased, and towards the end of a sixty-day experiment it had reached the initial values. Similar data were obtained for a number of other indices.

The results of our investigation allow us to conclude that in relation to the ability to withstand stresses, two phases of the change of reactivity of the organism in the process of hyposynamia are of importance: the first, lasting 15 to 20 days, is characterized by gradual reduction of the resistance to stresses and an increase of the extent of the reaction of the organism; the /53
second is a phase of stabilization, when reactions of the organism and the ability to withstand stresses are preserved at the level reached, approximately corresponding to the 15-20 days of hypodynamia.

Of great interest is the possibility of extrapolation of the data obtained during hypodynamia to conditions of the action of weightlessness in spaceflight.

According to reports of a number of investigators (Berry, 1966), reduction of orthostatic resistance was observed in manned spaceflights beginning with the thirty-fourth hour under conditions of weightlessness. After a fourteen-day flight, shifts of the orthostatic resistance appeared even less pronounced than after an eight-day flight.

Hence, during prolonged orbital flight, in the opinion of Berry and other researchers, development of adaptation to conditions of weightlessness is observed in cosmonauts. In actuality, during flights of man and animals lasting many days, O. G. Gazenko, N. M. Sisakayan and others (1964) noticed the

effects of a certain adaptation to the action of weightlessness, evaluated according to behavioral reactions and the state of the important physical systems.

Thus, the experimental data which we obtained, as well as the literature, evidently support the possibility of adaptation of the human organism to conditions such as hypodynamia and weightlessness.

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CHANGE IN CERTAIN INDICES OF THE FUNCTION OF EXTERNAL RESPIRATION DURING THE ACTION OF G-FORCES

S. F. Simpura

ABSTRACT: In studies on human beings (53 persons) dynamics of changes in the basic indices of the function of external respiration during the action of "spike-like" type (9-16 units) transverse g-forces ($+G_x$) which differed in value, were studied.

Shifts in the system of respiration and gas exchange during the action of "spike-like" type g-forces confirm the disturbance of the oxygen balance in an organism, which are determined by the value of the maximally achieved action. During a stress having a value of up to 10 units changes in the respiration and gas exchange are insignificant, but during the action of g-forces having values from 11 to 16 units they are more pronounced.

The high efficiency of ventilation in the lungs and gas exchange in the initial state and during the action of g-forces led to the least great disturbances of respiration and could have been one of the factors which determined the high resistance of an organism to transverse g-forces

Disturbance to the rhythm of respiration (its lag of 10-25 seconds) under g-forces of up to 13 units did not cause significant shifts on the part of the function of respiration and gas exchange; during g-forces of higher than 13 units (up to 16 units) there was a marked decrease in the effectiveness of ventilation and gas exchange in the lungs during rotation.

At the present time it has been established that one of the leading factors /54 which limit the ability of man to withstand prolonged transverse g-forces is a disturbance of the oxygen balance in the organism.

Studies which were conducted earlier were intended to study this problem during the prolonged action of "plateau" type transverse g-forces, which differed in time and duration (Cherniak et al., 1961; Roger, Smedal, 1962; Steiner, Mueller, 1961; Kotovskaya et al., 1962; Barer, 1962; Kiselev, 1962; Barer, Golov et al., 1966, and others).

In this way we established distinct changes in the system of external respiration, which are expressed in an increasing frequency of respiration, which is especially significant with larger g-forces and in an increased reduction of its depth. The respiratory volume for small and moderate volumes of the effect (8-12 units) increases, and then decrease sets in, which reaches extremely low values for loads of 16-18 units. These studies indicate that shifts in the system of respiration are due to primarily by the action of a component g-force in the "chest-back" direction. As a result of compression of the chest by mechanical forces and limiting of its excursion, pulmonary ventilation is disturbed, and the redistribution of the blood occurring at this time in the lesser circulation leads to disturbance of the normal ratios of blood flow and alveolar air in the lungs, which results in a lack of oxygen in the arterial blood. The authors assume that the indicated shifts are a leading component in general pathogenesis of the disturbance of functions of external respiration and gas exchange in the lungs.

As far as concerns the study of respiration during the action of g-forces which gradually increase in volume, that is, "spike-like" type, this problem /55.
has hardly been studied at all in the literature, and the information which does exist relates primarily to the study of the rate of respiration (Volynkin, Vasil'yev, 1967; Kotovskaya, Yeshanov et al., 1966). Therefore, the aim of the present study was to study dynamics of the change of the basic indices of the system of external respiration and gas exchange during the action of spike-like type g-forces which differ in value.

Basic tasks of the investigation were:

--determination of the relationship of shifts in the respiration system to the value of the maximally achieved g-forces;

--determination of the effect of a disturbance of the respiration rhythm during g-forces in the form of its brief lag to the basic indices of respiration and gas exchange;

--study of the importance of the efficiency of ventilation in the lungs to the extent that the changes of these indices of respiration and gas exchange are expressed during "spike-like" type g-forces.

This report was made on the basis of an analysis of seventy-seven experiments with the participation of fifty-three test subjects who were twenty to thirty years old. The direction of the stress was "chest-back," and the reclining position included a slope of the back of the armchair of 80° from the vector of acceleration. The buildup gradient was 0.2 units/second. The maximum values of the g-forces reached were 9-16 units.

We studied the following indices of respiration: rate, respiratory volume, pulmonary ventilation, consumption of O_2 and elimination of CO_2, the coefficient of regeneration, the coefficient of oxygen utilization and the oxygen debt. Respiration was voluntary. All of the indices were corrected to normal conditions. Invesitgation of gas exchange was carried out in separate rotating sections: during the period of buildup of rotational speed from one to six units, from six to ten units, and from ten units to the maximum permissible values, and also during the centrifuge braking period. In this way, all of the rotary motion was conditionally broken down into five sections: three during the acceleration period and two during the deceleration period (from a maximally permissible value to six units and from six units to one unit). The basic indices of respiration were subjected to statistical analysis.

Dynamics of the change of the basic indices which characterize the function of external respiration during the action of g-forces with a value of up to sixteen units are given in Figure 1.

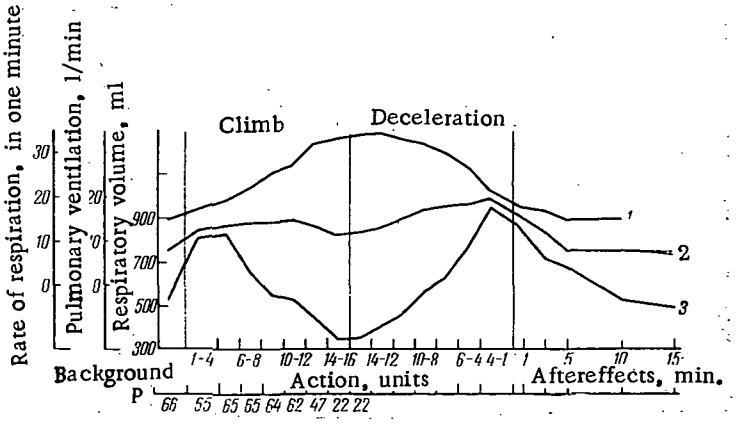


Figure 1. Nature of the Changes in the Indices of Respiration During the Action of Stresses Up to Sixteen Units. 1, Rate of frequency; 2, Pulmonary ventilation; 3, Respiratory volume; n, Number of tests.

In this scheme it is evident that the rate of respiration increased in relation to the growth of the stress. As the magnitude of the action seemed to increase this index increased more significantly. For instance the respiration frequency increased to 18.1 cycles/min during a stress of up to 6 units, as opposed to 14.6 cycles in the starting condition; this value was 25.6 cycles/min with a stress of 8 to 10 units, and it was 32.8 respirations per minute with a g-force of 14 to 16 units.

At the same time we notice changes on the part of the respiratory volume and pulmonary ventilation. From Figure 1 we can see that the respiratory /56
volume increased in comparison with the starting value then there was a stress of up to 6 units. Then, a gradual decrease of this index and its approach to the starting value set in along with the increase in the value of the action, and this became lower than the background level only after reaching a value of 12 units. Thus, the respiratory volume became equal to 358 ml during a g-force of 14-16 units as opposed to 525 ml before rotation.

Pulmonary ventilation charged differently. At the start of the rotation we noticed its gradual increase. Then, when the stress reached 8 units and up to 14 units, the well-known stabilization set in, when the pulmonary ventilation was maintained at a level approximately equal to 14 l/min. This level was maintained principally due to the rate of respiratory frequency, because the respiratory volume in this rotation period has already undergone a relative decrease. Then, in spite of the continuing increase in the rate of respiration, pulmonary ventilation also decreased somewhat and at g-forces of 14 to 16 units it was 11.2 l/min, although in this case it was higher than the starting background level (7.5 l/min).

In the starting period, in spite of the reduction in the value of the g-force, the frequency of respiration continued to hold at a high level and only after reaching a stress of 8 units did it increase noticeably. The gradual relative shortening and deepening of respiration led to an increase in pulmonary ventilation. The greatest changes of these indices began during the deceleration period after 4 units.

Under the conditions of our tests, regardless of the magnitude of the g-force achieved, the direction of changes of the indices mentioned was identical during the deceleration period for the centrifuge; however, the degree of their shifts varied (Figure 2).

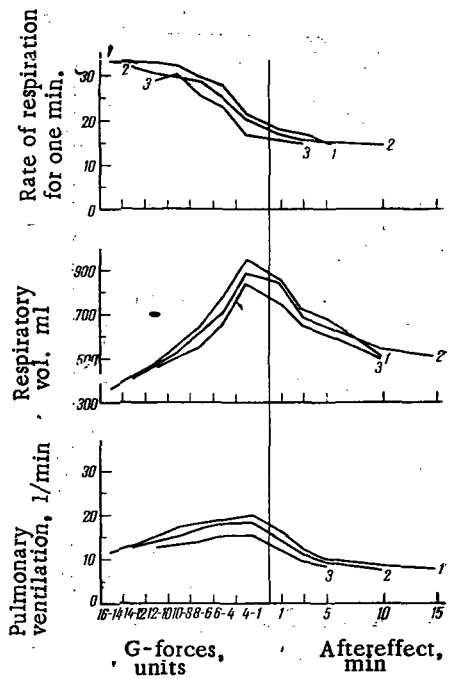


Figure 2. Change in the Basic Indices of Respiration During the Deceleration Period Through the Action of Spike-Like Stresses of Varying Magnitude. Maximum value of the stress is 16 units. (1), 14 units; (2), 12 units; (3).

While the respiratory volume was 840 / 57 mℓ, and the pulmonary ventilation was 14.9 ℓ/min for a stress of 4 units to 1 unit applied twelve times, for 14 units the values are 890 mℓ and 18.2 ℓ/min, respectively, and above 16 units the values are 950 mℓ and 19.6 ℓ/min. This difference was reliable ($P > 0.99$). Here, the degree of shifts in the respiration indices for this section of the rotation was determined by the magnitude of the g-force achieved and was in direct relationship to it.

Data for the consumption of oxygen and elimination of carbon dioxide confirmed the change of gas exchange during the action of the indicated g-forces (Figure 3). Because of the fact that the magnitude of the action has an effect on the nature of the shifts in the respiratory system, all of the persons examined were separated into five groups in relation to the maximally achieved g-force.

Figure 3 shows the dynamics of the changes in the gas exchange indices. In the first rotation section, when the g-forces were small (up six units), the consumption of oxygen increased almost by one and a half times in comparison with the starting values. In the next section, from six to ten units, a small decrease set in, which then continued as the value of the g-force

increased. However, until a g-force of thirteen units was reached, the consumption of oxygen continued to remain higher than the starting level. When there were larger values of this action a clear reduction of this index set in. Thus, for persons of the IV group, for whom the maximum value of the g-force was 15 units, the consumption of oxygen in this section for the stress value of 10 units to the peak value was 260 mℓ/min, but for persons of the V group it was 245 as opposed to 325 mℓ/min in the starting conditions.

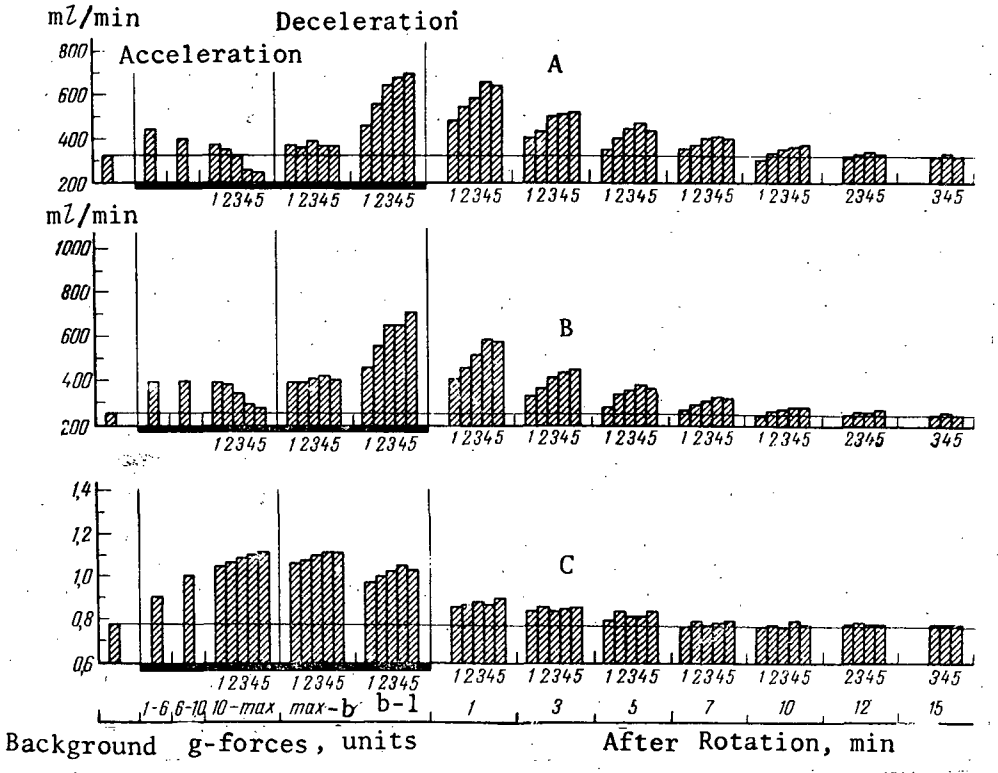


Figure 3. Dynamics for the Changes in Consumption of Oxygen (A), Elimination of Carbon Dioxide (B) and Respiratory Coefficient (C) During the Action of g-forces Which Vary in Magnitude and During the Period of Their Aftereffect. Groups, 1, (11.1 to 12.0 units); 2, (12.1 to 13.0 units); 3, (13.1 to 14.0 units); 4, (14.1 to 15.0 units); 5, (15.1 to 16.0 units).

Throughout the entire "acceleration" period the elimination of carbon dioxide was higher than in the starting background period. After a g-force of 10 units

was reached gradually decreased even more greatly than the value of the action /59
which was mentioned above. The elimination of carbon dioxide, although it had a tendency to decrease, nevertheless remained above the consumption of oxygen. Therefore the respiratory coefficient at this time increased and exceeded unity. It should be noted that similar shifts on the part of the consumption of oxygen, the elimination of carbon dioxide and the respiratory volume were noticed for "plateau" type stresses (Barer, Golov et al., 1966; Zechman et al., 1960; Steiner et al., 1961, and others).

The increase of these indices was characteristic for the deceleration period. It is true that during the first period of descent (from maximum values of the action to six units) the increase in the consumption of oxygen and the elimination of carbon dioxide was insignificant or was not present at all in comparison with the data obtained during the last part of the running of the centrifuge. The greatest growth came after a stress of six units was reached and depended on the value of the action achieved.

The analysis and comparison of pulmonary ventilation and consumption of oxygen showed that the reduction of these indices for different stress values which we noticed during the period of running the centrifuge and their significant increase at the end of its deceleration, and also during the aftereffects give indication of an oxygen deficit which was completely eliminated after stopping the action. The oxygen debt which was computed from the moment the centrifuge stopped is given in Figure 4. Inspection of this diagram clearly shows that the oxygen debt is determined by the value of the action endured and was in direct relation to it. This means that while after a stress of 9.1-10.0 units was reached it had an insignificantly small value of 87 ml, and a value of 854 ml with a stress 12.1-13.0 units, for a stress value of 15 and 16 units it increased on the average to 1,080-1,068 ml.

On the other hand these graphs show that the value of the oxygen debt in persons who withstood a maximum stress value of 15.1-16.0 units was approximately the same as for persons who withstood a stress of 14.1-15.0 units, which deserves separate consideration.

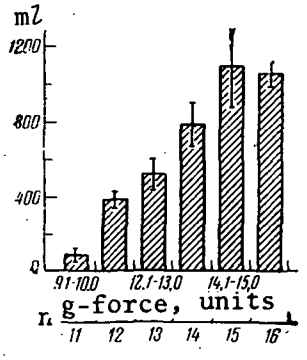


Figure 4. Change in the Oxygen Debt Depending on the Value of the Stress Achieved. n, Number of tests.

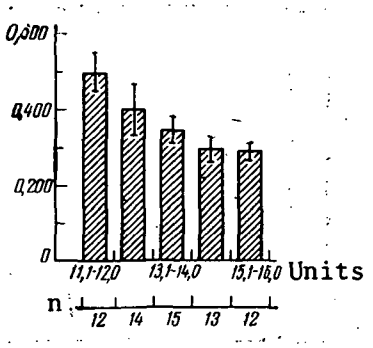
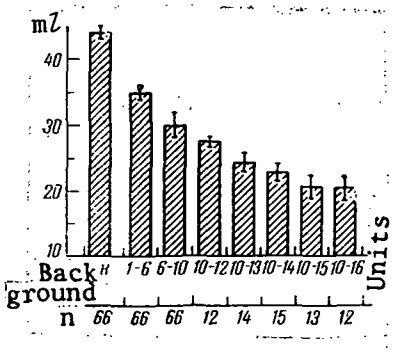


Figure 5. Coefficient of Regeneration Depending on the Maximally Achieved Stresses: Callouts, same as in Figure 4.



The coefficient of regeneration (CR--this is a ratio of the increase in oxygen consumption during work to the oxygen debt) is an index of the change in quality of the regulatory processes of the oxygen cycle. It is known that many authors (Belau, 1959; Damir and Pyatnitskiy, 1961, and others) attach great importance to this index. The increase of the CR is confirmation of the improvement in the reaction of an organism to physical stress, that is, the level of a person's training; on the other hand, its decline indicates a worsening of the oxygen availability.

In our experiments the CR depended on the magnitude of the action. Figure 5 clearly shows that a steady reduction of the coefficient of /60
regeneration is observed along with an increase in the magnitude of the stress. The shifts confirm the increase of the oxygen debt, which predominated over the increase in the oxygen consumption during rotation.

Our concept of the effectiveness of ventilation and gas exchange in our experiments is obtained from the change in the coefficient of oxygen utilization, that is, according to the amount of oxygen in ml which is absorbed in the lungs from one liter of ventilated air. It is known that the value of this index depends on the conditions

Figure 6. Coefficient of Oxygen Utilization for Different Sections of the Rotation Depending on the Maximally Achieved G-forces. Callouts, same as in Figure.4.

of diffusion of oxygen in the lungs, on the effectiveness of ventilation, and also on the perfection of coordination between pulmonary ventilation and circulation in the lungs. When this coordination is in disorder, the coefficient of the oxygen use is reduced, which confirms the worsening of ventilation effectiveness. Its increase is an index of the best use of ventilated air.

The data from our investigations show that when the pulmonary ventilation, respiratory volume and total oxygen consumption were at an adequately high level in the first sections of the rotation the coefficient of utilization was reduced. /61 Then it was even lower than the value of the stress achieved appeared to be, i.e., the relative effectiveness of ventilation of lungs reduced steadily along with the increase of the action (Figure 6). These shifts confirm the worsening of effectiveness of ventilation during stresses. Pathogenically, this may be due to certain disorders of hemodynamics in the pulmonary circuit which cause the change in the normal relationships between aeration and pulmonary blood flow.

After the action of stresses the regenerative period is characterized by the elimination of the oxygen debt and by elimination of carbon dioxide which is excessive in comparison with the starting background level. This led to reinforcement of pulmonary ventilation, to increase in frequency and deepening of respiration. As can be seen in Table 1, the regeneration of oxygen consumption, pulmonary ventilation and other indices of respiration did not occur at the same time.

TABLE 1. TIME OF REGENERATION OF INDICES OF EXTERNAL RESPIRATION AND GAS EXCHANGE AFTER THE ACTION OF VARYING STRESSES (IN MINUTES).*

Group	Stresses, units	Rate of resp.	Resp. vol.	Pulmon. ventil.	Consump. of O_2	Elimin. of CO_2
I	11,1 — 12,0	5	5	5	6	8
II	12,1 — 13,0	7	6	7	8	9
III	13,1 — 14,0	9	10	10	11	12
IV	14,1 — 15,0	11	11	11	13	14
V	15,1 — 16,0	9	6	9	11	12

*Translators note: Commas indicate decimal points.

Thus, the rate of respiration, respiratory volume and pulmonary ventilation reached the starting level somewhat more quickly than the gas exchange indices. It should also be noted that a lengthening of the time of regeneration of these indices was noticed

along with the value of the action which was endured. For example, for test subjects who withstood a maximally reached stress of 11.1-12.0 units (I group) normalization began within 8 minutes, but for persons who withstood a greater magnitude of stress action (III group) it took 12 minutes.

In addition, it should be recognized that for test subjects of the V group who withstood a stress value of 15.1-16.0 units the regeneration time for the indices of respiration was even less than for test subjects of the IV group, and it was the same in its duration as for test subjects of the III group after stresses of 13.1-14.0 units. In analyzing this material it was found out that /62 this applied to more than the recovery period.

In relation to the above we think it is necessary to make separate decisions on results of the change in indices for the system of external respiration in test subjects of the V group, for whom the resistance to stresses seemed greater, but for whom disorders of respiration and gas exchange were less.

At least two assumptions should be considered here: the high resistance to g-forces and the relatively smaller shifts in respiration might be due first of all to the high degree of conditioning of persons in this group to the action of g-forces and secondly, to the individual peculiarities of the respiration function, i.e., to its higher efficiency. It is from these positions that we have considered the results of the investigation of the V group.

Composition of the test subjects was the following: of the eight persons, two had repeatedly undergone the action of transverse g-forces and could be considered adequately conditioned test subjects. The remaining six persons were rotated in the centrifuge for the first time and not more than two-three times. Thus, the greater part of the test subjects in this group had not been conditioned to the action of transverse g-forces. Later we considered the problem of determining the peculiarities of external respiration in persons of this group both during the conditions of rest and during the action of g-forces. Then we compared them with data for test subjects of the remaining groups.

Analysis of the material showed that test subjects in group V in the starting condition differed from persons of the other groups by the fact that air respiration was less frequent and deeper, and the amount of oxygen consumed

in the utilization coefficient was higher, i.e., the individual efficiency of ventilation and gas exchange of air in the lungs was somewhat higher than for the other persons. This difference was reliable ($P > 0.999$). Later, during running of the centrifuge, the indices of external respiration and gas exchange also confirmed the improved use of oxygen from the ventilated air and the improved regulation of the oxygen cycle during the action of stresses. Therefore, the oxygen debt, the coefficient of regeneration and the normalization time for the test subjects in V group differed from similar changes in other persons.

Consequently, it is possible to assume that these differences in the external respiration system, i.e., its relatively fewer disturbances, in persons of this group are determined by the individually higher efficiency of ventilation of the lungs both during the starting condition and during the action of stresses, which in turn might be one of the factors which determine the higher resistance of the organism to the action of transverse g-forces.

At the present time it has been determined that difficulty of respiration during the action of transverse stresses is largely conditioned by malfunctioning of coordination of the respiratory movement, i.e., by the absence of a constant respiration rhythm, and by its frequent delays (Cherniak et al., 1961; Clarke, 1959, and others). Therefore the problem of studying a number of indices of external respiration in a group of persons who had quite evident disorders of rhythm in the form of respiration delay with a duration of ten to twenty-five seconds during the rotation process was subjected to special consideration. /63

Analysis of the material showed that in circumstances when the changes arose during g-forces of up to thirteen units, shifts on the part of the indices which characterize the function of respiration and gas exchange were insignificant or almost the same as when these disturbances did not exist. However, if disorders of rhythm were encountered for larger stresses, the degree of manifestation of the changes was greater than for persons examined with the same magnitudes of action but without disorder of the respiration rhythm. Comparison of the changes was made for the oxygen debt, the coefficient of regeneration and the coefficient of oxygen utilization (Table 2).

TABLE 2. CHANGES IN THE OXYGEN DEBT (OD), COEFFICIENT OF REGENERATION (CR) AND THE COEFFICIENT OF OXYGEN USAGE (CU) DURING THE ACTION OF G-FORCES WITH DELAY OF RESPIRATION (DR) AND WITHOUT IT (AVERAGE VALUES).

Group	Stresses, units	OD			CR			CU from 10 to limit		
		No DR	DR	Δ	No DR	DR	Δ	No DR	DR	Δ
I	11,1 — 12,0	386	405	+19	0,462	0,439	—0,023	27,4	27,7	+0,3
II	12,1 — 13,0	562	534	—28	0,430	0,385	—0,045	24,1	24,7	+0,6
III	13,1 — 14,0	720	1069***	+349	0,346	0,270**	—0,076	23,4	21,1***	—2,3
IV	14,0 — 15,0	999	1329**	+330	0,283	0,202*	—0,081	21,4	20,4	—1,0
V	15,1 — 16,0	1066	1166***	+100	0,270	0,228**	—0,042	20,9	19,5	—1,4

Note: Authenticity of the differences was determined by comparison with data without respiration delay. (*< 0.999; **< 0.99; *** < 0.95);

Note: Commas indicate decimal points.

It is known that during a test with holding of breath, changes which occur in an organism (increase of intrathoracic pressure, a drop of the intake capacity of the chest, etc.) can lead on the one hand to a decrease in the entrance of blood to the right side of the heart and then into the arterial system, and on the other hand to an increase of partial pressure of CO_2 and reduction of the O_2 pressure in the alveoli which causes hypoxemia and hypercapnia.

Respiration delay during muscular work makes these shifts worse. In addition, they begin earlier and are conditioned more quickly than during rest by the use of oxygen and the accumulation of CO_2 in the alveolar air. Accord- /64 ing to data of M. Ye. Marshaka (1961), a decrease in the oxygen saturation of blood when holding the breath during muscle activity begins after 5-10 seconds, but begins only after 25-30 seconds when holding the breath in a state of rest.

Consequently, it is possible to assume that under conditions of our tests holding the breath in combination with the action of stress, against a background of significant muscle tension, leads to larger shifts in the system of

external respiration and gas exchange than during rhythmic respiration. The results of our experiments actually confirm this position.

Thus, our material showed that shifts arise in the system of respiration and pulmonary gas exchange during the action of "spike-type" g-forces. We established that efficiency of ventilation and gas exchange in the lungs during the action of stresses depended on the magnitude of the action. This is due to changes in the pulmonary circuit, which lead to disorders of interrelations between pulmonary ventilation and pulmonary blood flow which cause oxygen deficiency in the arterial blood (Kiselev, 1962; Barer, Golov, et al., 1966; Steiner, Mueller, 1961; Nolan et al., 1963, and others).

Consequently, the phenomena of hypoxia which arise during the action of g-forces lead to formation of an oxygen deficit in the organism which is only eliminated during the aftereffect period and depends on the magnitude of the action.

The study of the nature of shifts in the respiration system at different times of rotation enabled us to discover that elimination of the oxygen debt under conditions of our tests began in the deceleration period of the centrifuge and ended after its halting. In our investigations the maximum value of the oxygen debt as computed after stopping of the centrifuge was somewhat greater than one liter (with extreme values of 845-1,622 mℓ) and was noticed after the action of g-forces having a maximum value of 15 to 16 units.

We also determined the value of efficiency of ventilation and gas exchange in the starting condition and when testing the ability of man to stand transverse g-forces.

In our tests we found a relationship in shifts of the respiration system to disorder of its rhythm (respiration delay). When these disorders arose before a g-force value of thirteen units was reached the changes were insignificant. In cases when the value of the stress was higher than thirteen units, shifts in the respiration system were noticeably higher.

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EFFECT OF ACCELERATIONS ON REACTIVITY OF THE GASTRO-INTESTINAL TRACT TO PHARMACOLOGICAL AGENTS

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and L. A. Tyutin

ABSTRACT: In roentgenographic experiments on white rats, the effect of transversely oriented accelerations on reactivity of an organism to pharmacological agents which act primarily on the gastro-intestinal tract were studied. A change is observed in the sensitivity of the rats to the action of acetylcholine and carbocholine for a period of five minutes in the period of aftereffect of accelerations having a value of twenty.

In earlier studies (P. V. Vasil'yev, V. Ye. Belay et al.,) it has been /65
shown that the action of transversely oriented acceleration causes a change in reactivity to narcotics, to cardiovascular agents, and to respiratory analeptics. This fact justifies the study of sensitivity of an organism to other medicinal agents during the aftereffect of acceleration.

At the present time is has been firmly established that after the action of accelerations on the organism of humans and animals clear changes in the secretive and the motor-evacuatory function of the stomach and intestines occur (I. N. Khazen, K. V. Smirnov et al.,). The nature of the function of disorders described by the authors allows one to assume that at this time a change in reactivity of an organism to pharmacological agents can also be ob- /66
served, which act primarily on the gastro-intestinal tract.

The study of these problems is the object of the present work. We used fifty-eight white rats as experimental animals and we separated them into three series of tests according to the agents which were being studied. In the first series we studied peculiarities of the action of atropine sulfate, acetylcholine hydrochloride in the second and carbocholine in the third on the motor-evacuatory function of the gastro-intestinal tract. There were four groups of animals in each series. The first and second groups were intact,

but the third and fourth groups were exposed to transversely oriented accelerations ($+G_x$) on a centrifuge with a radius of 4.2m. The acceleration was 20 g, the time of action was five minutes, and the rate of acceleration was 0.2 g/sec.

A conclusion regarding the nature of changes of the motor-evacuator function of the stomach and intestine due to the effect of medicinal agents and stresses was made on the basis of an analysis of survey X rays which enabled us to evaluate the speed of the passage of barium sulfate which was administered to the rats of all the groups in the form of a 5 mm quantity of 50% aqueous suspension. The animals of the second and fourth groups were simultaneously given subcutaneous injections of the pharmacological agents under study along with the barium sulfate. Roentgenological studies were made on the day of the action of the accelerations and also on the third, seventh, tenth, twelfth and fourteenth days following. The distribution of the animals according to groups and certain conditions of the experiments are given in the table.

DISTRIBUTION OF ANIMALS BY GROUPS AND SERIES OF TESTS

Series	Pharmacological agent	Dosage of prep. mg/kg	No. of rats	Distribution by groups			
				intact		with acceleration	
				1st	2nd*	3rd	4th*
I	Atropine	0.4	20	5	5	5	5
II	Acetylcholine	0.02	20	5	5	5	5
III	Carbocholine	0.02	18	4	5	4	5

*Groups which were given pharmacological preparations.

Evacuation of the suspension of barium sulfate from the stomachs of intact animals in the first group began in the first minutes after its administration. After 3-3.5 hours the stomach was normally completely free of the contrast mass which had completely filled the small intestine by this time.

Pronounced disorders of the tonus and motor-evacuatory functions of the /67
gastro-intestinal tract developed in all of the rats due to the effect of

acceleration (third group). We also noticed the rapid appearance of severe pyloric spasms and at times we also noticed a spasm of the antral section of the stomach which fluctuated in duration from 30 minutes to four hours in different rats. Later the pyloric spasm was repeated periodically. Evacuation of the barium sulfate suspension from the stomach was retarded in the majority of the animals for four to six hours, and in isolated cases remainders of the barium mixture were detected in the stomach of rats even twenty-four hours after its administration (Figure 1).

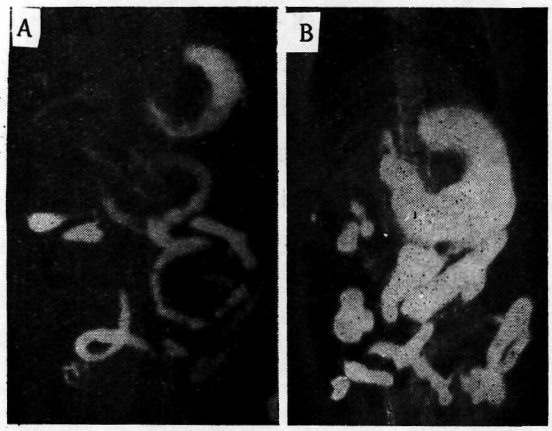

Figure 1. X-rays of the Gastro-Intestinal Tract of Rats Three Hours After Administration of Barium Sulfate. A, First group (intact, control); B, Third group (after action of acceleration) — pyloric spasms, evacuation disorder, dystonia of the small intestine.

We also noticed the phenomenon of an abrupt disorder of the tonus in the small intestines of the animals: primarily hypertonia of the intestinal loops with the presence of spasmodic sections; passage of the barium mixture in the intestine was normal or slightly accelerated. Normalization of the tonus and motor-evacuatory functions of the gastro-intestinal tract began about ten-twelve days after the action of the acceleration.

The data we discovered on the effect of accelerations on the functional condition of the gastro-intestinal tract of experimental animals agrees with the results of investigations of Britton et al., 1946), who noticed a delay in the evacuation of food from the stomach of rats, and also with the data of a number of other authors (P. M. Suvorov, T. V. Volkov, A. P. Mukhina, V. Ye. Potkin), who had observed disorders of the motor functions of the stomach both in man and in animals after the action of acceleration. /68

After the injection of atropine following the action of acceleration we observed changes in the tonus and evacuatory-motor functions of the stomach and intestine, which was similar to the changes during the action of individual accelerations only. In particular we noticed a delay in evacuation of a contrast mass from the stomach of four to six hours (Figure 2). A prolonged pyloric spasm occurred periodically. In the small intestine we noticed phenomena of dystonia and dyskinesia. These changes were retained to the ten-twelfth day after the action during dynamic observation, but their magnitude gradually decreased.

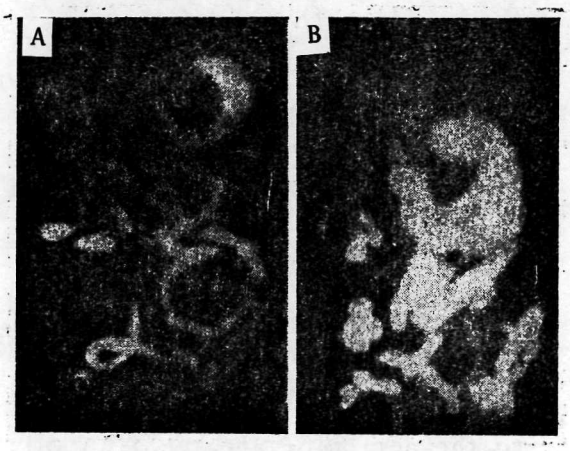

Figure 2. Roentgenograms of the Gastro-Intestinal Tracts of Rats Five Minutes after Administration of Barium Sulfate. A, Second Group (intact) - noticeable delay of evacuation after injection of atropine; B, Fourth Group (injection of atropine after the action of acceleration) — pronounced disorder of the motor-evacuatory function.

We were not able to detect the nature of the effect of atropine in the dosage which we used on the functional condition of the gastro-intestinal tract of rats after the action of acceleration in relation to the unidirectionality of the changes due to the effect of these factors.

After administering acetylcholine to the animals we noticed an accelerated evacuation of the barium sulfate suspension from the stomach by 1.5 to 2 hours in comparison with rats in the control group. The peristalsis was powerful, and the passage of the barium through the small intestine was accelerated.

After administering the acetylcholine following the action of accelerations on the animals we obtained data which were basically similar to the changes which we detected in rats which had been subjected to stresses without subsequent administration of pharmacological preparations (Figure 3). The specific action of the acetylcholine

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on the gastro-intestinal tract began to appear only on the tenth day and was manifested completely on the fourteenth day.

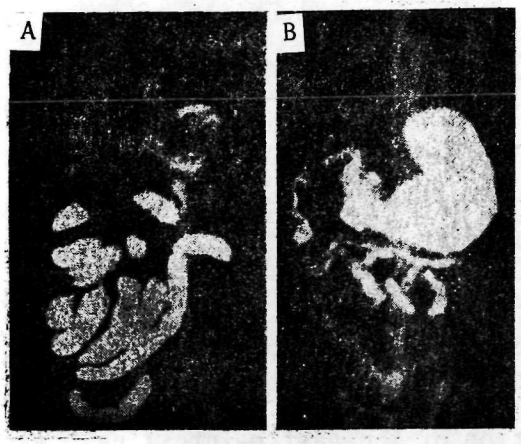

Figure 3. Roentgenograms of the Gastro--Intestinal Tract of Rats Two Hours After Administration of Barium Sulfate. A, Second group (intact) — noticeable acceleration of evacuation after administration of acetylcholine; B, Fourth group (administration of acetylcholine after the action of acceleration) — pronounced disorder of the motor-evacuatory function.

Because acetylcholine is a medium which promotes the transmission of excitation from cholinergic nerves to the affected organ innervated by them, we may assume that after the action of acceleration lasting ten to fourteen days suppression of the excitability of the cholinergic nerves of the gastro-intestinal tract is observed. This suppression is so pronounced that the applied dosage of acetylcholine does not cause any activity of the gastro-intestinal tract for 1.5 to 2 weeks.

In other words, the reactivity of the gastro-intestinal tract to the action of actylcholine following the action of accelerations changes substantially.

Vigorous evacuation of the contrast mass from the stomach usually began very quickly after administration of carbocholine to the rats; however, evacuation slowed down later and complete emptying of the stomach occurred in the normal amount of time. The tonus and peristalsis did not change. Movement of the contrast mass in the intestines was accelerated. After the action of acceleration and subsequent administration of carbocholine in the rats we noticed only /70
a brief pyloric spasm, and complete emptying of the stomach occurred after the same period of time as in the intact animals (Figure 4). Tonus of the small intestine was increased in the first two hours and then returned to normal. The phenomena of dystonia were not present. Movement of the barium mixture in the intestine was not disturbed.

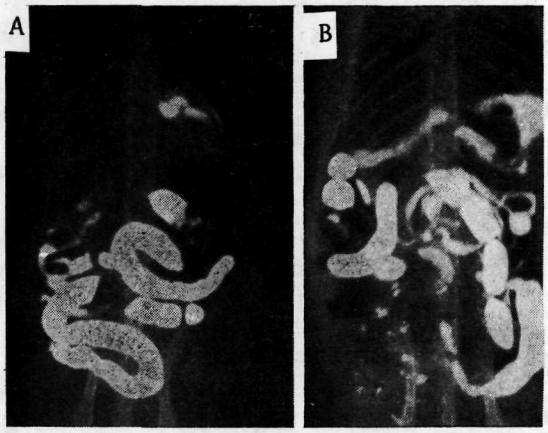

Figure 4. Roentgenograms of the Gastro--Intestinal Tract Three Hours after Administration of Barium Sulfate. A, Second Group (intact) — slightly accelerated evacuation of the contrast mass from the stomach after administration of carbocholine; B, Fourth Group (administration of carbocholine after the action of acceleration) — normal rate of evacuation of barium sulfate from the gastro-intestinal tract.

Subsequently, administration of carbocholine after the action of accelerations on the rats showed a clear normalizing effect on the functional condition of the gastro--intestinal tract. Complete normalization began on the third day after the action of stresses.

Our data show that after the action of acceleration the reactivity of the gastro-intestinal tract of rats to acetylcholine and carbocholine is substantially changed. Carbocholine has a predominantly normalizing action on the tonus and motor-evacuatory function of the stomach and intestines.

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CHANGES IN THE NUCLEI OF LIVER CELLS DURING THE ACTION OF TRANSVERSE STRESSES ON ANIMALS

Ye. F. Kotovskiy and G. A. Kosolapov

ABSTRACT: Karyometric study of the nucleus of liver cells of intact white rats and rats subjected to the action of stresses was performed with a value of 25 units for 10 minutes in the direction of chest-back. A reduction in the volume of the nuclei and the nucleoli one and two hours after the action of stresses was found. There was a reduction in the amount of nuclei of class K_3 six and seven hours after the action of stresses, but there was an increase in the number of large nuclei of classes K_4 and K_8.

Simultaneously the mitotic activity increased by eight to nine times and the amounts of small nuclei of class K_1 increased. A slight hemorrhaging in the liver which caused destruction of sections of the parenchyma was noticed.

In works devoted to the study of effect of stresses on the histological /71
structure of organs there are practically no data relating to the condition of the nuclei of cell elements. However, by controlling protein syntheses, which include enzymes in particular, the nuclei of cells determine the vital level and the functional activity of the cells. Therefore, the study of nuclei will deepen our understanding and add to our concept of the condition of cells as a whole.

In the present work we have made a karyometric study of the nuclei of liver cells (hepatocytes) of intact animals and animals subjected to one-time action of stresses having a value of 25 units for ten minutes in the chest-back direction. Four of the twelve white rats weighing 110-115 g, which participated in the experiment, were subjected to karyometric study. The limited number of animals was due to the considerable labor involved in the given method. We prepared liver prints for the karyometric studies which were carried out one, two, six and seven hours following the action of overloads. The prints were set in carnoit fluid for fifteen minutes and then placed in a freshly prepared solution of gallocyanin (chrome alum) for forty-eight hours. In addition, the /72

livers of all of the animals were treated by general morphological and by certain histochemical methods: nucleic acid by gallocyanin and methyl green with pyronine, proteins (α-amino acids) by alloxan reaction, glycogen by Schick reaction and fats by sudan.

Measurement of the nuclei and nucleoli in the prints was made along two diameters by a screw ocular-micrometer (objective 90, ocular 15). We measured 100 nuclei in each print and the number of nucleoli in them. The volumes of the nuclei and nucleoli were calculated according to the formula $V = \frac{\pi}{\sigma}B^2L$. In order to standardize the results of the calculation we squared the smallest diameter for the nucleus and the nucleolus. We took a logarithm for the absolute value of the volume of the nuclei and nucleoli, and the division of the nuclei and nucleoli into classes was made by the logarithmic method.

The results were represented in the form of histograms and then we made variation curves (graphs) according to these data. In addition, we studied the distribution of nucleoli in the corresponding classes of nuclei. It was not our goal to study the true dimensions of the nuclei because they are considerably flattened in the prints and, consequently, appear to have somewhat greater dimensions than in actuality. We were interested in the relative dimensions of the nuclei and the fluctuations in the classes of nuclei both in their relative values and in their frequency distribution.

Before presenting the data which we obtained it must be pointed out that the dimensions of the nuclei which were determined by the karyometric method are one of the reliable morphological criteria for evaluating the functional condition of cells according to a number of cytologists (Brashe, 1960; Brodskiy, 1966; Khesin, 1967). By the aid of this criterion we can detect changes in the activity of organs during the action of different factors.

The results of our investigation are presented in the form of combined graphs in which the variation curves of the volumes of nuclei, the variation curve of volumes of nucleoli and the distribution of nucleoli in the corresponding classes of nuclei are presented.

For intact animals (Figure 1) the variation curve of the volumes of nuclei of hepatocytes has three peaks, which correspond to the three classes of nuclei

with the following average indices of volumes: 600 μ^3 in class K_1, 1,250 μ^3 in class K_2, 2,500 μ^3 in class K_4. Judging from the graphs the most numerous are cells of class K_1 with cells of classes K_2 and K_4 following.

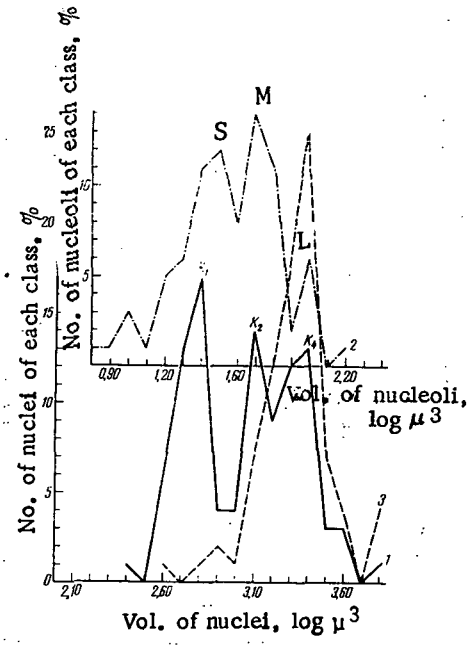

Figure 1. Variation Curves for Volumes of Nuclei (1), Nucleoli (2), and Frequency Distribution of Nucleoli (3) in the Liver of an Intact Animal. Classes of cells: K_1, K_2, K_4; Dimensions of nucleoli: S, small; M, medium; L, large.

The variation curves for the volumes of nucleoli also have three peaks, each one of which determines the dimensions and numbers of nucleoli which we have arbitrarily named small, medium and large. As can be seen on the graph, /74 the first and second peaks are higher. This confirms that the average and small nucleoli are the predominant ones in the nuclei of liver cells of a control animal. The distribution curve for nucleoli in the corresponding classes of cells also indicates that the majority of nucleoli are in the nuclei of classes K_2 and especially K_4. According to one point of view, the size of one or the total size of all the nucleoli reflects the level of protein synthesis in the cell. On this basis it can be assumed that it occurs most intensively in hepatocytes with nuclei of classes K_2 and K_4 and less intensively in hepatocytes with nuclei of class K_1. The variation curves for the volumes of nuclei and nucleoli of the hepatocytes retain the three-peak shape one hour after stress (Figure 2).

However, it can be seen that both curves have shifted somewhat to the left, and the peaks which correspond to classes of nuclei K_1 and K_2 have dropped. Relatively lower peaks began to prevail on the graph which corresponded to the smaller nuclei and nucleoli. The decrease in the volume of the nuclei and nucleoli in a majority of the hepatocytes and also a certain reduction in the

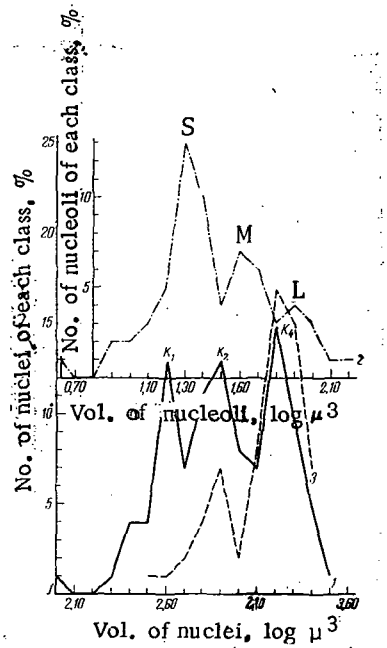

Figure 2. Variation Curve for the Volumes of Nuclei (1), of Nucleoli (2) and Frequency Distribution of Nucleoli (3) in the Liver One Hour after the Action. Callouts same as in Figure 1.

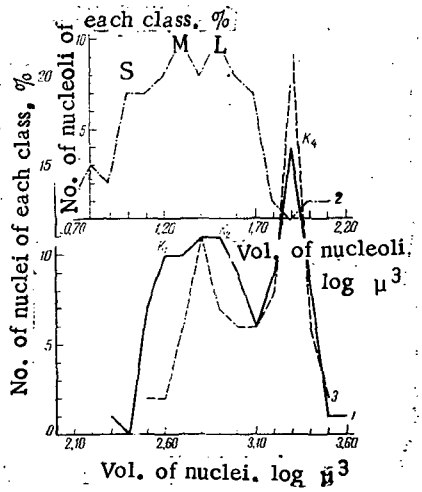

Figure 3. Variation Curves for the Volumes of Nuclei (1), of Nucleoli (2) and Frequency Distribution of the Nucleoli (3) in the Liver Two Hours after the Action. Callouts same as in Figure 1.

amount of protein and glycogen in the microscopic sections of the liver confirm the lower level of functional activity of the organ.

Two hours later, after the action of the stress (Figure 3), the variation curves for the volumes of nuclei and nucleoli remained depressed and shifted slightly to the left as before; i.e., toward the smaller nuclei and nucleoli of the hepatocytes. But it can also be noticed that the third peak which reflects the dimensions and number of nuclei of class K_4 rises a little and at 0.1 log shifts to the right in comparison with the preceding period. Simultaneously the number of nucleoli in this class of nuclei increases as is demonstrated by the graph of their distribution. The amounts of protein and glycogen in the microscopic sections of the liver remain at approximately their former levels.

It can be assumed that although the function of the liver at this time still has not reached the level of the control, a tendency toward its regeneration can still be seen, while the beginning of this process starts in the nuclear apparatus of the cell.

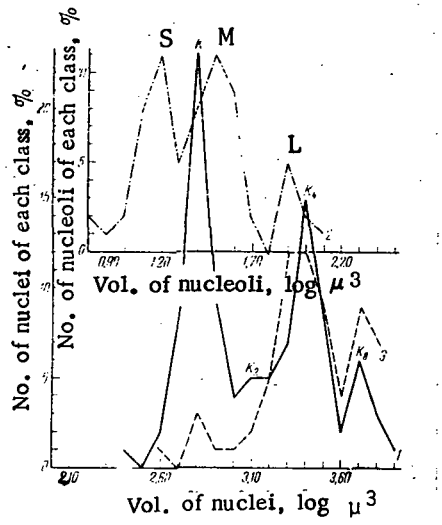

Figure 4. Variation Curves for the Volumes of Nuclei (1), Nucleoli (2) and Frequency Distribution of the Nucleoli (3) in the Liver Seven Hours after the Action. Callouts same as in Figure 1.

The shape of the variation curve for the volume of nucleoli changes six and seven hours after completion of the test with stress (Figure 4) due to the gradual leveling of the second peak (nuclei of class K_2), the increase of the first (nuclei of class K_1) and the third (nuclei of class K_4) peaks, and also due to the appearance of a fourth peak after seven hours, which corresponds to the larger nuclei of class K_8. The variation curve for the volume of the nucleoli shifts somewhat to the right, toward an increase in their dimensions, but the curve for the distribution of the nucleoli indicates that their numbers increase in the nuclei of classes K_4 and K_8.

A slight increase in the amounts of nucleic acids and proteins is noticed in histochemical preparations of the liver, but the amount of glycogen remains lower than in the controls. An important circumstance is the increase /75 in the mitotic activity of the hepatocytes by a factor of eight or nine in comparison with the controls. It is possible that the change of ratios in the classes of cells and reinforcing of the protein synthesis condition the increase of mitotic activity of the liver.

Thus, the results of our test show that the nuclear apparatus of liver cells suffers during the action of stresses with a magnitude of twenty-five units for a period of ten minutes which is the maximum permissible amount for the given species of animals (almost 30% of the animals perished). A decrease of the dimensions of nuclei and nucleoli with preservation of structure indicates a reduction and change of the regulating phenomena on the part of nuclei

and the drop in the functional activity of the cells is a result of this. A similar condition in the liver in our tests was preserved for the first two hours after the action. A gradual increase in the dimensions of the nuclei (class K_4 and class K_8), the mitotic activity and the number of small nuclei (class K_1) began at this time.

Evidently the increase in dimensions of nuclei and mitotic activity are two stages of one process of cell division which leads to elimination of after-effects of the action of stresses. There was hemorrhaging in the liver which caused the destruction of small sections of the parenchyma. One result of the reinforcing of mitotic activity was the unavoidable increase in the number of young and less differentiated cells with relatively small nuclei (class K_1). Therefore, it is very likely that even at this time (after six-seven hours) the functional capacity of the liver still has not reached the control level. However, it does contain all of the necessary factors for complete structural and functional regeneration.

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PROBLEM OF THE INTERACTION OF ANALYZERS AND DEGREE OF VESTIBULAR REACTIONS TO THE ACTION OF EXTERNAL STIMULI

S. S. Markaryan

ABSTRACT: Three series of investigations on human test subjects in order to determine the efficiency of certain methods of inhibiting illusory sensations and postrotational nystagmus based on the action of sound, light and proprioceptive stimuli were conducted.

The data obtained show that the action of light of varying intensity (0.1; 100 and 260 lux) causes inhibition of vestibular nystagmus while the duration of the illusion of counter-rotation remains almost unchanged. During the action of sound with a frequency of 1,000 Hz and intensity of 100-110 db the duration of the illusion of counter--rotation is abbreviated and the amplitude and duration of post-rotational nystagmus are reduced. Muscle forces which are developed during manual and especially during postural dynamometry shorten the length of the illusion of counter--rotation and shortening of the length of the illusion becomes more pronounced along with an increase of the muscular forces. Such an effect should be seen as a consequence of the inhibiting reactions which occur in the cortical and subcortical formations and which develop according to the principle of negative induction at the time the external stimuli are acting.

In the flight of modern airplanes conditions are created in which pilots /76
may receive incorrect ideas (illusions) regarding the position or movement of the plane in space. From the works of A. P. Popov (1940), Ye. M. Yuganov (1955), F. D. Gorbov (1956), Ye. M. Yuganov and D. M. Zakhmatov (1958), G. L. Komendantov (1959), M. D. Yemel'yanov (1963) and other authors, it is known that the action of accelerations on the pilot during flight might be a cause of disorder of spatial orientation not only as the result of their great magnitude but also as a result of the total effect of the angular, Coriolis and rectilinear accelerations on the vestibular analyzer.

Illusions of banking, gliding, pitching up, turning and slipping are among the most frequent illusory sensations. The illusion of banking is the most frequent among them, followed by that of inversion of the airplane and finally

complete disorientation. The length of the illusions fluctuates from several seconds to one minute and longer. Examination of large groups of fighter pilots who were experienced in handling flights in complex weather conditions as well as young pilots and students (Graybiel, 1945, et al.) showed that illusory sensations in flight are observed not only among young pilots but also among experienced pilots. Pilots with limited experience and also persons who have had long breaks in their flying experience as a result of having had severe infectious or chronic diseases often experience illusions which last a long time and are difficult to suppress. A relationship between the emotional condition of the pilot and the onset of illusions has also been noticed. Thus, for regular pilots and student pilots who experience increasing excitation, unrest, fear or tension during flight, illusions are observed comparatively frequently. The variety of conditions of the external environment in which illusions during flight arise shows that a great number of factors may cause the onset of sensations and complicate the study of illusions. The onset of illusions during flight is conditioned by the disturbance of physiological interaction of the analyzers. It is known that rotational movements determine the stimulation of receptors of the semicircular canals, and may cause reflex reactions in the external muscles of the eyes.

During the transition to visual flight illusions frequently disappear because the visual perception of ground landmarks, if they reflect the true position of the plane in relation to the Earth and naturally to the horizon, constantly correct the vestibular, proprioceptive and other sensations of the pilot which are related to the action of acceleration. Sometimes illusions are expressed so clearly that the pilot ceases to believe the indications of his steering and navigational devices and attempts to correct the position of the airplane according to his sensations. This frequently leads to a prolonged lack of spatial orientation. /77

During a reduced visibility, insignificant stimulation of the labyrinth (during bankings of the airplane and turns of the head) may become a cause of pronounced illusions of a prolonged nature. Medvill, (1959) described an instance when a pilot turned the airplane at a rate of 300-400°/second due to an incorrect decision. The compensatory eye movements were not able to ensure

fixation of the glance on surrounding objects. In such instances a pilot cannot concentrate his attention on either his instruments or on external visual landmarks. He perceives them only in the form of alteration of light and darkness. The same author described the condition of pilots during a spin: "If the pilot's escape from the spin is retarded for more than fifteen to twenty seconds (about five turns at high rotational speed), the visual images become indistinct and 'abrupt', the speed of rotation seems to increase although it does not do so in actuality." According to observations of Graybiel and Hupp (1946) a post-rotational nystagmus of opposite direction arises after leaving the spin, which is connected with an apparent rotation of the field of vision (oculogyral illusion). This can distort the visual information of a pilot (Schindl, 1957) and lead to disturbance of orientation of the pilot as a result of contradictory signals on the part of the organs of vision and vestibular apparatus. In this instance the receptors of the vestibular apparatus are strongly excited due to the effect of angular accelerations which arise during the spin. In ~14% of cases this leads to flight emergencies and crashes (Bemding, 1959; Nuttall, Sanford, 1959).

One of the reasons for the disruption of spatial orientation (Popov, 1940; Parin, Bayevskiy, Yemel'yanov, Khazen, 1967, et al.) might be the turns of the head during specific maneuvers of the airplane as a result of which Coriolis accelerations arise. Bhatia (1957) studied the eye movements of a pilot during landing by the aid of a recording of corneo-retinal potential. It turned out that they might reflect the illusion of change of movement of the airplane in relation to the Earth. Pilots whose eye movements are uniform and constant are in a more advantageous condition during landing than pilots with less uniform eye movement. The author also noticed that during angular accelerations of an object at less than 80°/second the clear distinction was insured for slow eye movements, but at angular velocities of 80-150°/sec the rate, amplitude and velocity increased in repeated investigations due to the intermittent movements. /78

In studying the mechanisms of the development of spatial concepts in man, I. M. Sechenov discovered the role of the proprioceptive pulsation which accompanied each movement, in particular movement of the eyes for all spatial forms of vision. V. M. Bekhterev (1882) was the first one to show that pulses from

the receptors of the vestibular nerve reached not only as far as the nuclei of the medulla oblongata and the midbrain as well as the nuclei of the cerebellum, but also the cerebral cortex. Like I. M. Sechenov, he figured that an important role in spatial visual orientation is played by the proprioceptive pulses which accompany eye movement. The scientist especially emphasized that sensations which are basic to our sense of position of the separate parts of our body are the "chief culprits of our spatial perception."

I. P. Pavlov assigned the chief role to the analyzer of movement (among all the internal analyzers) in signaling at every moment the position of the body and the condition of all of its parts which participate in movement. By an analyzer, the physiologist meant not only the peripheral receptor but also the conducting path, the nuclei, and the cerebral cortex and assigned the greatest significance to the cortex in the activity of the analyzers.

E. Asratyan (1947) showed in direct tests that the central compensation of the vestibular disorders after disconnecting both labyrinths is accomplished by the cerebral cortex: he observed complete elimination of compensation as a result of removal of the cerebral cortex in dogs. Studies by I. S. Beritashvili (1953, 1959) were intended to clear up the role of the vestibular apparatus in spatial orientation of animals. In his experiments he established that orientation in space is accomplished by the effect of single labyrinthine receptors. Reflection in the brain of spatial relationships between objects in the environment, and also between individuals and objects is created on the basis not only of visual but also of labyrinthine stimulation which in this connection play a substantial role.

S. N. Khechinashvili (1958) compared the behavior of different animals after disconnecting the labyrinth. He often noticed that the more organized the animal, the more quickly compensation set in after disconnecting the labyrinth. On the basis of numerous studies, I. S. Beritashvili (1953, 1959) and S. N. Khechinashvili (1958, 1960) concluded that men and animals orient themselves in space chiefly as the result of labyrinthine pulsations, but other analyzers have a definite importance. On the basis of numerous experimental studies on animals E. Sh. Ayrapetyants and V. A. Kislyakov (1957), /79

V. A. Kislyakov and I. V. Orlov (1966) concluded that a prominent role in spatial orientation is played by the motor analyzer with the participation of the vestibular analyzer.

K. L. Khilov (1952) thought that the cortex of the brain was a principal regulator of the vestibular-somatic reactions, but is affected by factors of the internal and external environment.

V. V. Strel'tsov (1945) assumed that a cause of illusory sensations in flight is the increased excitability of receptors of the semicircular canals, the otoliths or their central representatives, and possibly the reduction of excitability of the other sections of the cortex of the brain. A. P. Popov (1940) explained the most frequent illusions of false banking under flight conditions by the fact that the vestibular apparatus is always in a condition of "working adjustment" and is related to the tonus of the musculature of the extremities in which small stimulations quickly accumulate. Evidently, the accumulation at such a critical moment is sensed so greatly that a pulse in the skeletal musculature is created by aid of the vestibular apparatus. A change of tonus in the skeletal musculature during the forward movement with comparatively small velocity and enforced prolonged sitting in a certain posture might become a source of banking illusion.

Ye. M. Yuganov and D. M. Zakhmatov (1958) consider the onset of illusory sensations during flight as a result of comparative predominance of processes of excitation in the cortical "representative" of the vestibular analyzer accompanied by a deficiency of inhibiting phenomena on the part of other analyzers, especially the visual. G. L. Komendantov (1959) explained the origin of spatial illusions by the disorder of the functional systemics of the analyzers. This author sets up three groups of causes which explain disorientation in flight. The first group is the limitation of the activity of one analyzer or the disturbance of the synthetic activity of the cortex which enters into the systemics. In the second group the disturbance of systemics arises secondarily and is due to weakening of the cortical compensation for disturbances to the function of subcortical sections of the central nervous system. And in the third group, as the author notices, damage to the functional systemics also arises secondarily as the result of difficulties in activity of the optical

analyzer (the impossibility of differentiating landmarks). Thus, the physiological cause of the origin of illusions must be considered a change of dynamics of the cortical processes which leads to malfunction of the relation between vision and other analyzers which participate in the formation of the concept of the spatial position of the plane.

Data in the literature show that illusions during flight represent a serious danger to the members of the flight crew and often cause air crashes. Study of the interaction of analyzers is of interest in searching for methods /80
of controlling illusions.

This process has already attracted the attention of physiologists and clinicians. It has already been convincingly shown that the extent and nature of the manifestation of vestibular reaction changes during the action of vestibular, optic, sound, olfactory and temperature stimuli on an organism.

The problem of the reflector interaction of analyzers underwent significant development in the works of L. A. Orbeli (1933), V. I. Voyacheka (1946), S. V. Kravkova (1948), V. N. Chernigovskiy (1956) et al. . The published data confirm the existence of a reflex relationship between different systems of analyzers. N. Ye. Vvedenskiy showed in 1897 that the sense of touch is increased due to the effect of light. N. A. Vishnevskiy (1936), Ye. N. Simnaovskaya (1946), K. Kh. Kekcheyev and A. A. Dubinskiy (1946) showed that the sensitivity of the cone apparatus of the eye is reduced during the action of sound at a frequency of 800 and 2,000 Hz.

Thus, N. A. Shurygin (1901), B. V. Tolokonnikov (1945), N. V. Timofeyev (1948), Ya. Z. Brailovskiy (1954) and others observed reaction of the pupil to sound stimulation. Ye. M. Belostotskiy and S. A. Ilina (1937) established the reduction of photosensitivity of the eye during stimulation of the vestibular indices and a reduction in the number of figures perceived according to shape and color during stimulation of the vestibular analyzer.

It is also known that receptors of the vestibular analyzer (ampullar radicles of the semicircular canals) are capable of reacting to sound stimulation. The experimental works of B. V. Tolokonnikov (1945), Tullio (1925), and others as well as the clinical observations of a number of authors

[Belogolovov(1925); Voyachek (1946); Brailovskiy (1954), and others] also confirm this. It is also known that sound stimulation under specific conditions led to reduction of the post-rotational nystagmus and the illusion of counter-rotation (Komendantov, 1933; Tolokonnikov, 1945; Min'kovskiy, 1952; Markaryan, 1959; Bazarov, 1964; Tullio, 1925). V. S. Nazarenko (1965) observed inhibition of the vestibulo-vegetative reactions during auditory stimulation of the olfactory and visual analyzers. Data in the literature confirm the theory of the location of the so-called sound receptor in the ampullar radicles of the semicircular canals. In the works of S. F. Shteyn (1909), K. L. Khilov (1952), B. I Arapov (1961) it is shown that stimulation of the vestibular analyzer is reflected in the functional condition of other analyzers. S. P. Lapin (1946) observed reinforcement of olfactory acuity during weak otolithic stimulation. /81
The effect of stimulation of the olfactory receptor on the function of the vestibular analyzer was also studied by Ye. F. Yudan (1941), V. S. Nazarenko (1965), K. V. Fokin (1967) and V. G. Bazarov (1967). According to the data of V. G. Bazarov the duration of illusion of counter-rotation decreased by an average of 7.8 seconds in 54.6% of the subjects tested under the influence of toluene. The nature of the olfactory reaction, according to work by O. G. Ageyeva-Maykova (1957), N. S. Blagoveshchenskaya (1962) and others depends on the functional condition of the cortex of the brain and other analyzers.

The effect of stimulation of the vestibular analyzer and the somatic reactions of pilots is reflected in the works of many authors (Kulikovskiy, 1939; Borshchevskiy, 1957; Medvill, 1959, et al.,), who have observed damage to coordination of movements to varying degrees. I. F. Tsion (1879) and V. M. Bekhterev established a connection between the vestibular analyzer and the skeletal musculature in animal experiments. Magnus (1924) related the redistribution of muscle tone to stimulation of the otolithic portion of the vestibular analyzer.

Experimental investigations on animals led S. N. Khechinashvili (1958) to conclude that the origin of pulsation during adequate stimulation of receptors of the semicircular canals leads to vestibular atony and nystagmus.

Important results were obtained by aviation physicians during the study of the interaction of the vestibular and motor analyzers. G. L. Komendantov

(1940) observed dizziness, nausea, quickening of the heartbeat as well as weakening of the muscle tone with subsequent reduction in the quality of flight operation in individuals with insufficient statokinetic resistance under flight conditions. V. A. Vinokurov, V. V. Levashov and A. I. Khromushkin (1946) noticed damage to coordination of movements in controlling an airplane during stimulation of the vestibular apparatus in pilots. The authors considered these changes a consequence of the reduction of muscle force.

I. Ya. Borshchevskiy (1957) described a change in quickness and accuracy /82
of firing depending on excitation of the vestibular apparatus.

Under conditions of brief weightlessness, Ye. M. Yuganov and coauthors (1960, 1963), while studying the interaction of the vestibular and motor analyzers of animals, discovered a reduction in the bioelectric activity of the skeletal musculature. The authors connected this with peculiarity of the function of the vestibular analyzer. The degree of damage to interaction between the motor and vestibular analyzers, as was shown in the works of K. L. Khilov (1952), P. L. Bareladze (1962), I. S. Usachev (1964) and others depended on the functional condition of the cortex of the brain; that is, on its exciting and inhibiting processes.

According to the observations of a number of authors (Platonov and Konovalov, 1955; Gorbov, 1956; Yuganov and Zakhmatov, 1958; Komendantov, 1962, et al.), even pilots with good flying background used their own "methods" to control illusory sensations during flight, and used different procedures: change in body position, shaking the head, muscle stretching, inducement of physical pain in the form of biting the lips, pinching themselves, etc. Of course, these methods for eliminating illusory sensations do not always work. Undoubtedly, these empirically applied methods are based practically on the interaction of the analyzers and are of interest for developing methods for the prevention of vestibular disturbances.

A current problem for aviation and space medicine is the search for methods of inhibiting illusions and nystagmus in flight. In this connection we studied the functional condition of the vestibular analyzer and certain aspects of its interaction with the auditory, proprioceptive and visual analyzers. The

problem of the effect of auditory, light and proprioceptive stimuli is closely connected to the problem of controlling vestibulo-sensory and somatic disorders in flight.

In order to determine the efficacy of certain methods of inhibiting illusory sensations and post-rotational nystagmus which are based on the action of auditory, light and propioceptive stimuli, we conducted three series of investigations.

In the first series we studied the condition of the vestibular analyzer and motor reactions to the action of an auditory stimulation of varying intensity. Sixteen persons participated in the experiment. Each of them was subjected to Barany-type rotation repeated four times in an electrically powered rotating armchair with intervals of three minutes. After the first rotation we determined the extent of nystagmus and the duration of illusion of counter-rotation (starting level). We fed a sound with a frequency of 1,000 Hz and /83
intensity of 100 db into the helmet headphones with a "ZG-2A" tone generator for five seconds during the second and third rotations immediately after stopping the armchair. In the first instance it was continuous but in the second it was pulsating in the form of five pulses (the length of each pulse was 0.25 seconds).

After the fourth rotation we fed a pulsating sound with a frequency of 1,000 Hz and intensity of 110 db for five seconds. The duration of the illusion of counter-rotation was indicated by the subject himself by pressing an electric marker which is connected to a chart recorder.

We studied the condition of the vestibulosensory and motor reaction during stimulation of the propioceptors in the second series of investigations. For this we used a manual and bench dynamometer which recorded the length of the illusion of counter-rotation. Fifteen persons participated in the investigation. Each of them was subjected to Barany-type rotation four times. The interval between rotations was five minutes. The test subject was told to exert a given muscle force by the aid of one of the indicated devices and determine the moment of disappearance of the illusion in the basic test immediately after the rotating was stopped. These tests were an attempt to examine the

specific habits of controlling illusory sensations which pilots attempt under flight conditions. The test subject was rotated with dark glasses and with his eyes closed in order to eliminate the effect of visual inhibition on the duration of the illusion of counter-rotation.

In the third series of tests we determined the effect of light of varying intensity on post-rotational nystagmus and the illusion of counter-rotation. Twenty healthy men were observed in this experiment. Each test subject was subjected to Barany rotation on two occasions (with intervals of five to six days), four times on each occasion with five-minute breaks between rotations. After rotation the post-rotational nystagmus was recorded on a four-channel electroencephalograph with an ink recording device. Electrodes were fixed to the outer corners of the eyes of the test subjects. The curve obtained reflected the duration of nystagmus, the total number of nystagmic shocks, and amplitude. We also calculated the length of illusion of counter-rotation, the beginning and end of which were set, based on the oral response of the test subject. The experiments were conducted in a dark room.

A light source was placed 50 cm in front of the test subject at eye level. In order to eliminate the visual perception of objects (especially in order to clear up the role of light in inhibition of illusion) the test subject wore special glasses with a liquid filter, into which we poured a latex[4] solution in a specific dilution.

Studies of the action of light at 0.1; 160; and 260 lux were conducted in /84
two variants. Twelve persons participated in each of them. In the first, we used a less concentrated solution of latex which enabled us to see separate objects (a luminous point) in an unoriented field, and in the second we used a more concentrated latex (0.5 ml of latex to 200 ml of distilled water and one ml of ammonia), as a result of which the test subject did not perceive surrounding objects when supplied with a light signal.

In all of the tests we studied the general condition of persons and their subjective sensations. We conducted 220 tests in all.

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[4]Disperse solution of synthetic rubber in water, which is formed during the process of emulsion polymerization.

Our investigations established that the duration of illusion of counter--rotation, independent of the nature of the sound signal being supplied, decreased by an average of 7.3 seconds in comparison with the starting value. The pulsating sound affects the duration of the illusion of counter-rotation more effectively than the continuous sound (Table 1). This agrees with the claim of the test subjects that the "illusion passes more quickly when supplied with a pulsating sound."

The sound also had an effect on the nature of the pulsed *rotational nystagmus*: rhythm was disturbed, a tendency to shortening of its duration was observed and there was a change in the amplitude (Figure 1).

TABLE 1. CHANGE OF DURATION OF ILLUSION (IN SECONDS) OF COUNTER--ROTATION DURING THE ACTION OF SOUNDS.

Person tested	Control	Continuous sound of 1,000 Hz and 100 db	Δt	Control	Pulsating sound of 1,000 Hz and 100 db	Δt	Control	Pulsating sound of 1,000 Hz and 110 db	Δt
A-v	17	12	-5	15	8	-7	18	9	- 9
Zh-v	13	10	-3	14	9	-5	12	5	- 7
M-v	14	9	-5	9	3	-6	16	8	- 8
P-v	10	6	-4	14	9	-5	12	5	- 7
Kh-n	12	7	-5	15	9	-6	13	6	- 7
N-v	9	6	-3	13	8	-5	15	7	- 8
B-y	21	16	-5	18	12	-6	22	9	-13
K-v	16	12	-4	17	12	-5	15	7	- 8
Ts-v	26	20	-6	21	13	-8	23	12	-11
N-y	8	6	-2	11	7	-4	9	4	- 5
R-ko	12	9	-3	14	10	-4	11	5	- 6
L-*n*	7	5	-2	5	2	-3	8	3	- 5
G-v	16	11	-5	19	12	-7	17	8	- 9
Ye-v	29	23	-6	26	17	-9	30	17	-13
D-y	21	16	-5	18	11	-7	22	12	-10
U-v	13	9	-4	12	6	-6	16	7	- 9

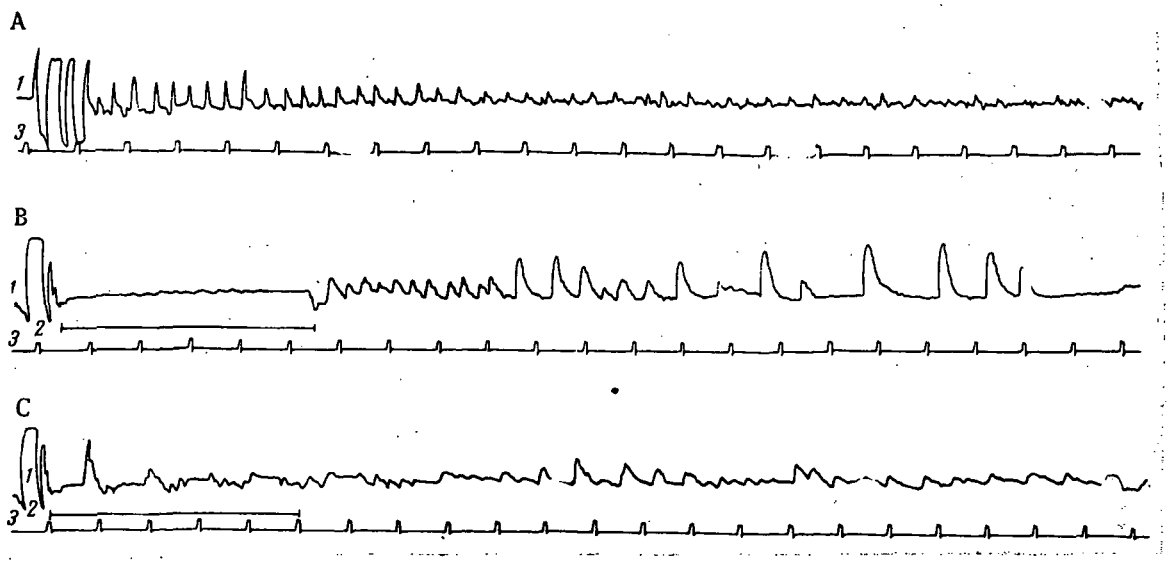

Figure 1. Nature of Pulsed Rotational Nystagmus of Test Subject G-v During the Action of Sound Stimulus. A, Control; B, During the action of continuous sound stimulation; C, During the action of interrupted sound stimulation; 1, Nystagmogram; 2, Stimulus mark; 3, Time mark, seconds.

As can be seen in the nystagmogram above nystagmic pulses decreased /86 noticeably during the action of sound stimulation. When the sound was discontinued they arose again but with a disturbed rhythm.

In studying the action of sound on the manifestation of vestibular reaction we also considered the individual evaluation by the test subjects of the signals being given. Some perceived the continuous sound with frequency of 1,000 Hz and intensity of 100 db best of all, while others (the majority) perceived best the pulsating sound with the same characteristics. The pulsating sound with a frequency of 1,000 Hz and intensity of 110 db had an unpleasant effect on the test subjects, even though the duration of illusion of counter-rotation decreased significantly.

The use of manual and postural dynamometry by the test subjects reduced the duration of the illusion of counter-rotation by an average of 6.2 seconds in comparison with the starting value. The greatest reduction in the illusion

was noticed during the application of machine dynamometry (Table 2). The test subjects noticed that as the forces which are applied increase the duration of the illusion of counter-rotation becomes less and vice versa. If muscle tension is weakened, the illusory sensations are again reinforced (especially in persons with increased vestibular sensitivity), but to a lesser degree than at the beginning of the test. After stimulation of the vestibular analyzer with subsequent application of manual and especially postural dynamometry the test subjects felt better than during the control investigation. The motor and partially the vegetative reactions were also expressed to a lesser degree.

Results of our investigations with the action of light on the visual analyzer showed that the degree of manifestation of the indices of post-rotation rotational nystagmus changes while the duration of illusion of counter-rotation remains almost unchanged. The duration and amplitude of post-rotational nystagmus in comparison with the control values decreased two-fold and even more during the action of light of 160 and 260 lux. These changes were pronounced to a lesser degree during the action of light of 0.1 lux through a more concentrated solution of latex (Table 3). The post-rotational nystagmus varied more greatly during the action of a light source of 160 and 260 lux on the visual analyzer independently of the concentration of the latex solution. /87

TABLE 2. THE DURATION OF THE ILLUSION OF COUNTER-ROTATION DURING MANUAL (A) AND POSTURAL (B) DYNAMOMETRY (IN SECONDS).

Test subject	Control	A	Δ t	Control	B	Δ t	Test subject	Control	A	Δ t	Control	B	Δ t
I-v	8	5	-3	10	7	-3	P-v	13	8	-5	10	2	- 8
A-n	4	2	-2	6	2	-4	Khr-v	8	5	-3	12	6	- 6
M-v	15	11	-4	13	5	-8	P-y	24	18	-6	27	13	- 14
N-v	14	10	-4	17	5	-12	Z-y	17	11	-6	15	6	- 9
R-v	7	4	-3	9	2	-7	Sh-v	13	7	-6	12	4	- 8
Sh-v	5	2	-3	5	1	-4	K-k	25	18	-7	28	12	- 16
N-kh	7	4	-3	10	3	-7	B-v	10	7	-3	13	4	- 9
G-v	10	6	-4	14	5	-9							

TABLE 3. TIME FOR THE POST-ROTATIONAL NYSTAGMUS INDEPENDENT OF THE LIGHT INTENSITY (IN SEC.)

Light, lux	Rotation	Latex solution	
		Less concentrated	More concentrated
Control	1st	27(52)*	28(63)
0.1	2nd	15(33)	25(53)
160	3rd	12(26)	13(30)
260	4th	12(26)	15(34)

*In parentheses — number of nystagmoid movements.

In Figure 2 we have presented curves for the nystagmus of test subject D-ko in order to illustrate the relation of the nature of the post-rotational nystagmus to the light intensity which acts through eyeglasses with a more concentrated solution of latex.

It can be seen from the graph that the post-rotational nystagmus before the action of light on test subject D-ko lasted 26.5 seconds, but the number of nystagmoid movements was 49. During the action of a light source of 0.1 lux on the visual analyzer the post-rotational nystagmus was abbreviated to seventeen seconds, but the number of eye movements was reduced to forty-two; at 160 lux these values were respectively 11.5 and 25, and at 260 lux they were 9 and 26. The peak of the nystagmic curves on the oscillograph chart was sharply reduced in all of the cases. Thus, the time, frequency and amplitude of the post-rotational nystagmus were sharply abbreviated due to the effect of the light.

We changed the duration of the light action in order to analyze more deeply the nystagmic reactions. The nystagmus was delayed during the action of light of 160 and 260 lux on the visual analyzer through eyeglasses with more concentrated latex solution. Three to five seconds later, after visible cessation /89 of the nystagmus, we turned off the light and the nystagmus appeared again, while the number of nystagmoid movements did not decrease, but at times even increased in comparison to the control investigations. We have presented data of the action of light of varying intensity on nystagmus in test subject B-n as an example in Figure 3.

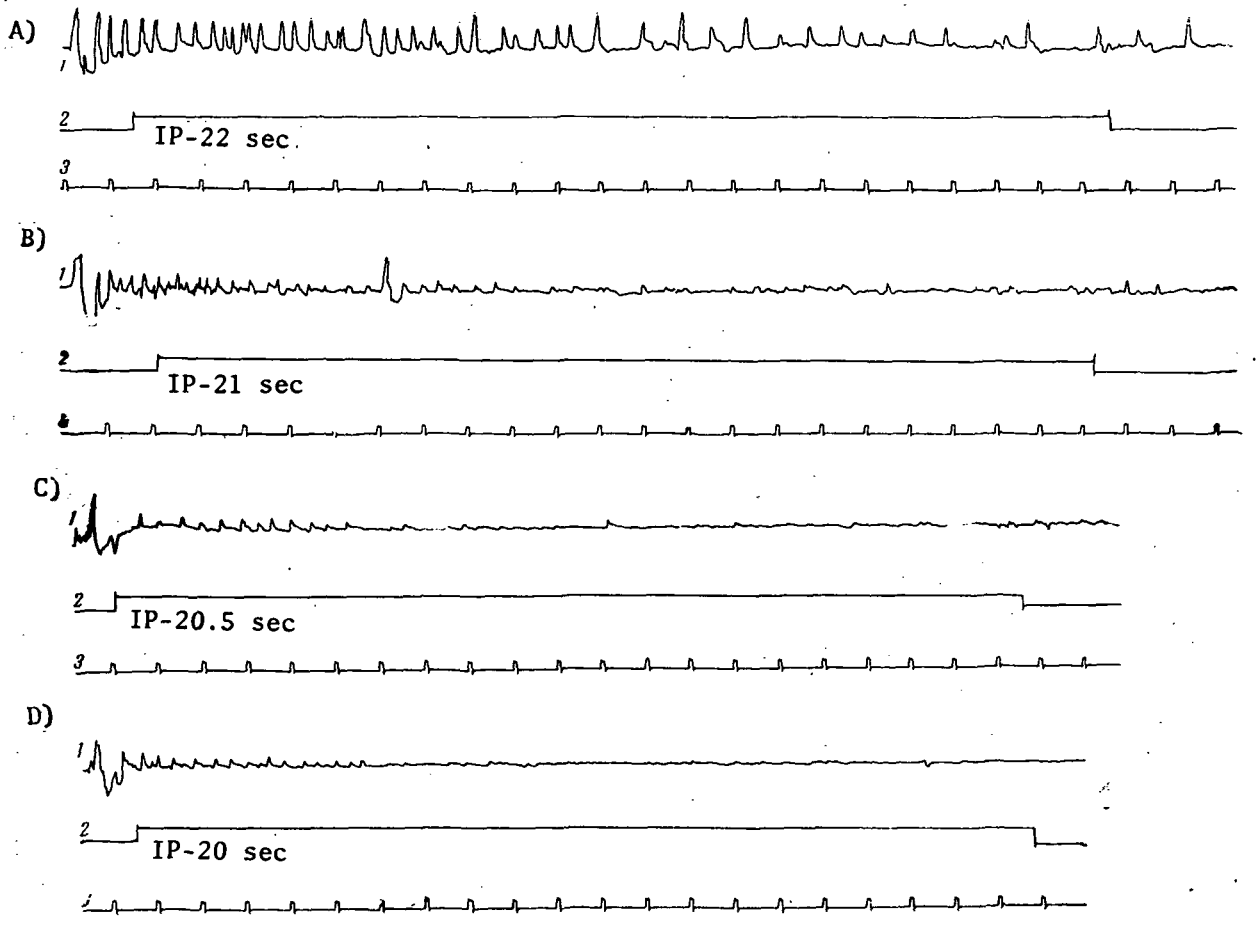

Figure 2. Nature of the Post-Rotational Nystagmus for Test Subject D-ko During the Action of Light Stimulation of Varying Intensity. A, Control; B, During the action of light of 0.1 lux; C, The same — 160 lux; D, The same — 260 lux. IP, Duration of illusion of counter-rotation; 1, 2, 3, See Figure 1.

In the control tests the post-rotational nystagmus lasted twenty-five seconds, but the number of nystagmoid movements was forty. During the action of light of 160 lux the peak of the nystagmus sharply decreased, and its duration was seventeen seconds, but the number of movements was twenty-seven. Three seconds later, after disappearance of the nystagmus, the light was turned off, and the nystagmus appeared again and lasted six more seconds (thirteen nystagmoid movements).

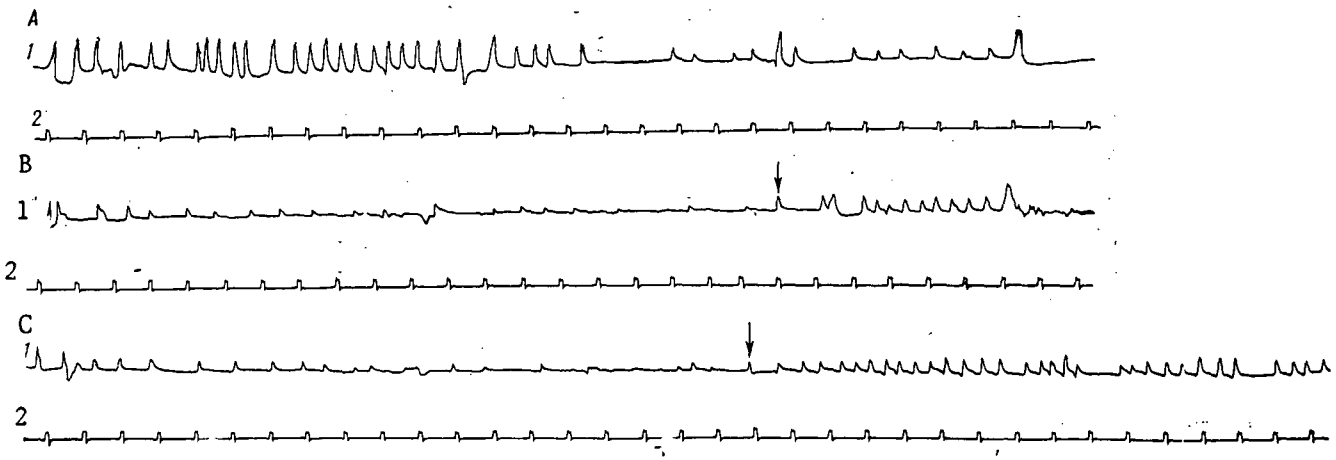

Figure 3. Renewal of Nystagmus for Test Subject B-n after Stopping the Light Stimulation. From top to bottom: post-rotational nystagmus (1) and time (in seconds) (2); A, Control; B, During the action of light of 160 lux; C, The same 260 lux; Arrows, Moment of cessation of light action.

At 260 lux the duration of nystagmus decreased to thirteen seconds (seventeen movements). As soon as a straight line appeared on the chart in place of the nystagmic peaks, we maintained the interval for five seconds and turned off the light. The nystagmus appeared again, this time with greater amplitude than during the control investigation, and the number of movements was thirty-seven. Similar data were obtained for the remaining test subjects.

Thus, the reflex interaction of analyzers was clearly shown in investigations of the action of sound, light and proprioceptive stimuli on the function of the vestibular analyzers. During the action of sound and light stimuli we observed a reduction of post-rotational nystagmus. The renewal of nystagmus after turning off the light is evidence of the continuing activity of the vestibular centers during the aftereffect of adequate stimulation. Evidently, in this case the functional peculiarity of the central sections of the vestibular analyzer of maintaining excitation for a long time is manifested.

During the action of light this vestibulosensory reaction, like the illusion of counter-rotation, varied somewhat differently. It remained stable in the control tests and during the action of light independently of the light intensity. The fluctuations of the duration of the illusion of ±1-3 seconds which we noticed might be related to errors made in the experiments.

Both light and sound had an effect on the nature of the post-rotational nystagmus, its rhythm, amplitude and duration. Whereas the illusion of counter-rotation did not change during light stimuli, its duration was abbreviated during the action of sound stimulation and during muscular exercise. The reduction of the vestibular reactions (nystagmus, illusion of counter-rotation) due to the effect of sound and light stimulation and goal-oriented muscular exercise should be viewed as the braking reaction which occurs in the cortex of the brain and which develops according to the principle of negative induction between the central cortical formations of the vestibular and other analyzers. The experimentally obtained results confirm the effectiveness of procedures which are used by some pilots for suppressing illusory sensations and are further supported by well-known experimental data.

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On the basis of the classical teaching of A. A. Ukhtomskiy regarding the dominance of nerve processes in the cerebral cortex, K. L. Khilov (1952) suggested the hypothesis that by means of stimulation, for example, of an olfactory, sound or other receptor, it is possible to achieve dominant excitation of the corresponding cortical centers. According to the law of negative induction, this will enable inhibition of pulses which reach the cortex from the vestibular analyzer. This position is confirmed and expanded in the experimental investigations conducted by us.

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CONDITION OF THE VESTIBULAR ANALYZER IN DOGS AFTER PROLONGED IRRADIATION IN SMALL DOSES

P. I. Kumets

ABSTRACT. The functional condition of the vestibular analyzer in sixty dogs, some of which are subjected to continuous radiation action in overall doses of 25-225 rads/ /year were evaluated. Six dogs were given medical-preventive agents. The method of quantitative evaluation of the functional conditions of the vestibular analyzers showed an increase in excitability of the spatial analyzer by 35-40% in animals which had been irradiated with a total dosage of 225 rads/year. Observed is a tendency towards decrease of excitability and increase of reactivity in the group of animals which had been irradiated with a dose of 225 rads/ /year and which had received medical preventive agents.

The wide use of ionizing radiation in industry and medicine has made the /93
problem of limits of sensitivity of the nervous system to the action of ionizing radiation extremely important.

Information on the biological action of irradiation during evaluation of radiation danger on prolonged spaceflights is especially important. On the one hand, the possibility of maintaining normal activity of the central nervous system brings us closer to solving the general biological problem of life in space, and on the other hand the knowledge of peculiarities of the reactivity of the nervous system in relation to extreme environmental factors, data on the limits of resistance and its endurance enable us to solve practical problems in the creation and improvement of biotechnical systems and to develop means and methods for improving the resistance of an organism.

According to prevailing concepts disorders of spatial orientation may arise in cosmonauts during flight. As is known, spatial analysis is achieved by the complex integrated activity of a whole set of analyzers: vestibular, visual, auditory, motor, skin, interioceptive and others. An important role /94
in carrying out spatial orientation is played by the vestibular analyzers. In

spaceflight the vestibular analyzers function under conditions which are uncommon for Earth. Weightlessness in combination with sufficient stimuli (accelerations) changes the afferent impulsation, while the evolutionary complex interrelations between the cupular apparatus and the otoliths are disturbed. In addition, under conditions of prolonged spaceflight ionizing radiation will constantly influence the nonauditory labyrinth. Therefore, it is important to have information on the radiosensitivity of the cupular apparatus in evaluating the radiation danger in prolonged spaceflights.

From the data in the literature it is known that under total irradiation of an organism and localized radiation effects functional and morphological changes on the part of the nonauditory labyrinth are observed. At this time, in the opinion of some authors, there is an increase of excitability of the spatial analyzer (Moskovskaya, 1958 et al.), while in the opinion of others, there is a suppression of it (Petelina, 1959; Titov, 1957, et al.). These data were obtained chiefly during abrupt total actions, while in some of the works accurate characteristics of the physical conditions of the radiation action and amounts of the doses of ionizing radiation are not presented, sufficient stimulus for the semicircular canals of the labyrinth was not used, and the effect of the dependence on the radiation dose was not investigated.

It should be especially emphasized that up until now we have had very limited data on the condition of the nervous system during chronic irradiation of dogs in small doses.

Therefore, it appeared important to us to obtain information on the nature of the functional changes of the vestibular analyzers under conditions of prolonged constant irradiation in such doses as might cause only reversible changes in the activity of the nonauditory labyrinth, doses which would be threshold ones for the function of the analyzer and finally in doses which would simulate to some extent conditions of concrete radiation conditions of prolonged spaceflight.

By the methods of quantitative evaluation of the function of the vestibular apparatus we were able to obtain statistically reliable data which

characterized disruption of the activity of the semicircular canals in dogs during prolonged action of small doses of ionizing radiation on the organism.

We shall present the result of the functional condition of the vestibular apparatus in dogs after their continuous chronic irradiation for a year with different total doses. The material presented is part of an overall study of the observation of 200 dogs which were subjected to irradiation by gamma rays /95 under conditions which simulate possible radiation conditions during prolonged spaceflights as to the amount and intensity of doses (Grigor'yev, Markelov, Popov, et al., 1968).

The doses of chronic irradiation for the different groups of animals were 25, 75, and 150 rads/year (table). In addition we studied the effect of chronic irradiation (75 rads/year) in the experiment in combination with repeated abrupt actions of 50 rads three times. Some of these animals were given a vitamin--amino acid complex, which contributes to an increase of the overall nonspecific resistance and radioresistance of an organism (Pogozkin et al., 1967), regularly once every two months for two weeks before acute irradiation for preventive and therapeutic purposes.

CONDITIONS OF IRRADIATION OF DOGS AND THE VALUES FOR EXCITABILITY OF THE ANALYZER.

Group	Conditions of irradiation	Total dose, 1 yr, rads	No. of dogs	Excitability threshold (stop-signal) °/sec
I	0.07 rad/daily	25	6	8
II	0.21 rad/daily	75	6	8
III	0.51 rad/daily	150	6	8
IV	0.21 rad/daily + abrupt irradiation of 50 rads 3 times	225	6	5
V	0.21 rad/daily + abrupt irradiation of 50 rads 3 times + treatment with preventative complex	225	8	7.5
VI	Control	--	26	8.5
VII	Control with use of medical-preventative complex	--	2	7.1

There were sixty dogs in the test, of which thirty animals were subjected to chronic irradiation in the indicated doses and thirty dogs were controls. The study of the condition of the vestibular analyzer was conducted in a sound insulated, shielded and darkened chamber on a VU-2 device, which enabled us to obtain a wide range of angular accelerations (Grigor'yev, Bokhov, 1961). We evaluated the threshold sensitivity and reactivity of the vestibular analyzer to an adequate stimulus as well as certain vestibulo-vegetative reflexes, and evaluated the interaction of the visual and vestibular analyzers. We rotated the animals with a uniform angular velocity for two minutes, with subsequent stopping of the machine ("stop-stimulus"). At the moment we stopped, a negative acceleration began, which was sufficient stimulus for the semicircular canals; the angular velocity gradually increased as did the negative acceleration when /96 the machine was stopped before appearance of the first nystagmic movement of the eyes appeared in the dogs. Later we evaluated the reactivity of the analyzer ("reactivity test"). We rotated one dog for two minutes at various velocities, beginning with small above-threshold values (10, 30, 60, 90°/sec) and ending with maximum values (120, 150, 180°/sec). After stopping the unit, i.e., after the action of the negative angular acceleration of varying value, we determined the duration of nystagmus, the rate of respiration and the pulse.

We turned on a light stimulus of 2,000 lux for ten seconds and recorded the nystagmus while testing the interaction of the visual and vestibular analyzers upon stopping the unit (which was rotating at a velocity of 180°/sec). Previously we had used an identical adequate stimulation of the vestibular analyzer without a light stimulus.

In dogs which had been subjected to chronic exposure at doses of 25 and 75 rads/year, the excitability thresholds were 8°/sec, respectively (the threshold value of the stimulus was characterized by the angular velocity of the machine before it stopped) (see table). In a group of animals with a total dose of 225 rads/year, the threshold decreased by 35-40%.

The use of an adequate stimulus gradually increasing in value (angular velocity of 10, 30, 60, 90, 120, 180°/sec) with subsequent stopping of the unit showed that the vestibulo-somatic reflex did not change in the group of animals given a dose of 25, 75, 150, 225 rads/year. The vestibulograms for these

animals were practically identical. The test of the interaction of analyzers also showed that this parameter was similar in all of the animals examined. Thus, the use of the "reactivity test" did not show any differences in values for the vestibular somatic reflex in healthy and irradiated animals. One should note the interesting fact of the less pronounced changes in threshold sensitivity in the group with irradiation at a total dose of 225 rads/year plus nonspecific treatment in comparison with a similar group which had not received a medical-preventative agent.

In the group of animals which had received medications there was no abrupt decrease in the excitability thresholds, but we did observe an insignificant increase in the reactivity of the vestibular analyzer: the duration of the nystagmus increased, and the angle of inclination of the vestibulograms became greater. The test of the interaction of analyzers in this group of animals showed a reduction in the effect of the visual analyzer on the vestibular.

Results of our investigations indicate an increase in sensitivity of the spatial analyzer in dogs with a total radiation dose of 225 rads/year. We noticed a certain positive effect of the vitamin amino-acid complex on the resistance of the vestibular analyzer to an adequate stimulus. Evidently the changes in the excitability of the analyzer are functional, inasmuch as the reactivity of the spatial analyzers did not change in the group of animals with doses of 25, 75, 150 rads/year and in the group of dogs with total radiation dosage of 225 rads/year. The interaction of the spatial and visual analyzers in these groups of animals also did not change. /97

The material presented showed the involvement of the nervous system during the reaction of an organism to chronic irradiation in small doses. In this connection it seems proper to us to draw a certain analogy in the ratio of the known phase of excitation of the nervous system as observed during the effect of acute radiation in such small doses as 0.05-0.5 rad (Grigor'yev, 1963). We observed an increase of excitability of the cortical terminal of the visual analyzer in the first hours after acute action. A. V. Sevan'kayev (1963) and L. A. Tabakova (1968) found an increase of excitability of the vestibular analyzer two hours after total irradiation in doses of 50 and 100 rads in different animals (rabbits).

In considering the above, the certain functional changes in the part of the vestibular analyzer which we have noticed may be evaluated as a condition of the phase of excitation of the central nervous system which corresponds to the first stage of expressed compensatory mechanisms.

In addition, the increase of the excitability of the vestibular analyzers during the action of small doses of radiation again indicates the correctness of considering the cupular apparatus as the critical organ in evaluating radiation danger in prolonged spaceflights.

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III. ARTIFICIAL ATMOSPHERE AND ITS EFFECT ON THE ORGANISM

CONDITION OF METABOLISM DURING PROLONGED STAY OF MAN IN A SMALL ENCLOSURE WITH CYCLICALLY CHANGING ATMOSPHERE

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ABSTRACT: The nitrogen and water-salt metabolism in two test subjects who stayed for 35 days in a small enclosure with a cyclically changing atmosphere were studied.

There is a tendency to reduction of the negative nitrogen balance during the action of hypoxic gas mixture with some excess of carbon dioxide which probably is conditioned by the increase in activity of the respiratory muscles. At this time a change in the structure of the water metabolism is noted.

The necessity of simulating emergency situations due to the failure of /98
human life support regeneration systems in closed quarters with limited space is evident. Above all it is important to determine the humanly tolerable limits of a changed gas environment and to determine the amount of reserve time. In addition, it is necessary to establish tests for determining the possibility of reducing the unfavorable action of hypodynamia and hypokinesia by the aid of an active change in the gas environment. This opinion is shared by a number of authors (Volynkin, 1968; Lynch et al., 1967).

It is well-known that a number of physiological and biochemical shifts (Popov, 1962; Zhdanova, 1965; Mogendovich, 1961; Lampusov, 1962: Taranov and Parfenova, 1965) are observed among healthy persons under conditions of hypodynamia caused by strict bed rest, by water immersion, and by prolonged stay in a small enclosure.

Metabolic changes are expressed in the negative nitrogen and electrolytic balance (Popov, 1962; Syzrantsev, 1967; Cuthbertson, 1929; Graveline et al., 1961). In addition, the composition of the body changes and there is a

reduction in muscle force and endurance (Zhdanova, 1965; Lampusov, 1962; Taranov, Parfenova, 1965; Helander, 1957.

There is one theory that the oxygen deficit creates a necessary functional load and it also reduces the negative effect of the reduced physical load. It has been shown that acclimatization to altitude causes an increase in the volume of circulating blood, an increase in the amount of erythrocytes and hemoglobin, and promotes retention of protein and calcium salts in the organism. /99 During hypodynamia we have observed reversely oriented shifts. The inventors of an "active" artificial atmosphere have suggested the hypothesis of the effectiveness of a small increase in the concentration of carbon dioxide in cabin air. It is possible that the combination of a small oxygen deficiency with an excess of carbon dioxide leads to a more pronounced physiological effect and simultaneously reduces the unfavorable action of oxygen insufficiency.

In this paper we have studied nitrogen and water-salt metabolism in two test subjects who were placed in a SBK-48k pressure chamber for thirty-five days. The composition of the gas environment changed.

We used a cyclical seven-day chart for fluctuations in the parameters of the atmosphere. The given values for the parameters are presented in Table 1.

TABLE 1. DYNAMIC CHANGE OF THE PARAMETERS OF A GAS ENVIRONMENT IN A PRESSURE CHAMBER

	Days			
1st-2nd	3rd	4th	5th-6th	7th
Normal composition	PO_2 = 110 ± ± 5 mm Hg pCO_2 = std.	pO_2 = 110 ± ± 5 pCO_2 = 15 ± ± 3 mm Hg	Normal composition	pO_2 = 320 ± ± 10 mm Hg

In all we conducted five cycles: four at normal temperature (21 ± 4°) and five at 28-29° with maximum permissible increase to 35°. The level of relative humidity was maintained within the limits of 60 ± 20%.

Limitation of the test subjects' muscular activity was made necessary by the crowdedness in the small space of the enclosure, which was occupied by one permanently unfolded sleeping place which the test subjects used as a working space during the day. The remaining free space was occupied at night by a second sleeping place, and part of the living quarters was occupied by research apparatus, food stores, and papers.

The physicians on duty noticed that the physical activity of subject Khr-v was less than that of subject F-v.

The test subjects were kept on a diet which contained products preserved by thermal processing (meat preserves in jars, soups and juices in tubes), by the method of sublimation drying (meat, dairy products) and by the method of thermal drying (baked goods), etc.

The daily rations contained (by calculation): 125-139 g of proteins, 104--108 g of fats, 320-330 g of carbohydrates. Their caloric value was 2,830--2,925 kcal. The amount of protein was determined by chemical means, and was 107-125 g. /100

In addition, they were given vitamins daily: 2 mg of A; 100 mg of C; 2 mg of B_1; 2 mg of B_2; 2 mg of B_6; 25 mg of E, etc. Water consumption was limited to 2 l of drinking water per day.

The test subjects' urine was collected each day during the experiment and its contents of total nitrogen, creatinine, ammonia, sodium, potassium, chlorine, calcium and phosphorus were determined. We determined the nitrogen balance and the assimilation of the protein in the food. We studied the actual nutrition and the water consumption. We determined the extrarenal water losses.

We used the seven-day cycle of active artificial atmosphere in order to thoroughly understand the action of the gas environment as a basis for classifying the material.

The elimination of total nitrogen in the urine for subject F-v (Table 2) was less than for subject Khr-v.

TABLE 2. EXCRETION OF TOTAL NITROGEN IN THE URINE (IN GRAMS) IN THE SUBJECTS FOR DAYS OF THE EXPERIMENT DEPENDING ON GAS ENVIRONMENT.

Test subject	By cycles	Days of cycle						
		1	2	3	4	5	6	7
F-v	First	12.0	17.9	21.3	7.3	15.1	16.6	22.2
	Second	18.6	22.0	21.5	16.3	17.8	16.4	15.5
	Third	18.5	17.1	17.8	14.6	18.9	15.3	17.9
	Fourth	17.9	13.6	19.7	18.7	19.5	20.4	15.8
	Fifth	14.7	12.6	12.9	14.2	16.1	14.0	16.2
	Average	16.3	16.7	18.6	14.2	17.5	16.5	17.5
Khr-v	First	22.6	18.8	20.1	25.6	43.2	24.0	17.8
	Second	11.6	22.6	20.3	18.4	18.1	26.7	9.7
	Third	19.1	20.7	19.8	17.3	28.2	14.2	18.4
	Fourth	20.5	20.0	28.8	23.9	16.7	20.3	23.4
	Fifth	27.5	20.4	19.7	16.0	17.2	14.6	16.8
	Average (M ± m)	20.3	20.5	21.7	20.2	24.7	19.9	17.3
		18.3± 1.46	18.6± 1.04	20.2± 1.15	17.2± 1.6	21.1± 2.7	18.2 ± 1.34	17.4± 1.14

The nitrogen losses in F-v rarely exceeded the limits of 20 g, which are normally accepted as the upper limit of the standard, but for Khr-v as a rule they were greater. Evidently, the reason for this difference should be attributed to the lesser amount of physical acitivity of Khr-v and his somewhat larger consumption of protein.

On the average, the excretion of nitrogen in the urine for both test subjects was less on days when there was an increased amount of carbon dioxide, but also on days when there was an increased amount of oxygen.

The total elimination of nitrogen in the urine and feces during the experiment exceeded its intake from the food which points to a negative nitrogen balance in the test subjects (Table 3). /101

The smallest negative nitrogen balance for both test subjects was noticed on the fourth day (deficit of O_2 + excess of CO_2), on the seventh day (excess O_2) and the first day (normal atmosphere). In test subject F-v the nitrogen balance was positive on the fourth day.

TABLE 3. NITROGEN BALANCE (IN GRAMS) FOR TEST SUBJECTS BY DAYS DEPENDING ON THE GAS ENVIRONMENT*

Test subject	By cycles	Days of cycle						
		1	2	3	4	5	6	7
F-v	First	+ 5.8	- 2.5	- 6.1	+ 9.9	- 0.6	- 1.8	- 5.0
	Second	- 3.6	- 7.0	- 4.9	- 2.9	- 4.0	+ 0.8	- 2.8
	Third	- 3.6	0	- 4.1	- 0.6	- 1.8	- 1.2	- 5.1
	Fourth	- 0.9	+ 1.5	- 5.1	- 2.7	- 4.4	- 5.6	+ 1.2
	Fifth	+ 0.8	+ 2.0	+ 4.2	+ 0.3	- 3.5	+ 1.4	- 3.2
	Average	- 0.3	- 1.2	- 3.0	+ 0.8	- 2.8	- 1.3	- 3.0
Khr-v	First	- 4.6	- 3.3	- 5.1	- 7.8	-27.7	- 9.2	- 0.2
	Second	+ 4.0	- 7.6	- 2.4	- 2.8	- 3.1	- 8.9	+ 5.9
	Third	- 4.6	- 3.4	- 4.7	- 2.5	-10.3	+ 1.3	- 3.6
	Fourth	- 2.7	- 4.5	-13.9	- 6.0	- 1.1	- 5.4	- 5.6
	Fifth	-10.2	- 5.6	- 2.0	- 0.5	- 2.4	+ 3.2	- 1.3
	Average	- 3.6	- 4.9	- 5.6	- 3.9	- 8.9	- 3.8	- 1.0
Average for two test subjects		-1.96	-3.04	-4.41	-1.56	-5.89	-2.54	-1.97

*Without calculation of the nitrogen loss in perspiration.

The decrease in the negative nitrogen balance on the fourth day attests to the high muscular tension; this increase in physical activity involves the respiratory muscles. On these days an increase of gas exchange in the test subjects was noted.

Evidently, during conditions of hypodynamia the increase in activity of respiratory muscles takes up a significant proportion of the total volume of the physical occupation, and has special significance in the initial period of adaptation of an organism to hypodynamia and weightlessness.

Data on the excretion of creatinine in the urine (Table 4) shows that we were not able to establish any kind of relationship of creatininuria to the action of the gas exchange. We also noticed a slight increase in the excretion of creatinine in the second half of the experiment for both test subjects. The /102 values for creatinine excretion fluctuated within normal limits.

TABLE 4. EXCRETION OF CREATININE IN THE URINE (IN GRAMS) FOR TEST SUBJECT BY DAYS OF THE EXPERIMENT DEPENDING ON THE GAS ENVIRONMENT. /102

Test subject	Gas composition by cycles	Days of Cycle						
		1	2	3	4	5	6	7
F-v	First	1.45	1.80	2.02	1.93	1.73	1.71	1.48
	Second	1.86	1.76	1.87	o.57	1.76	1.63	1.20
	Third	1.50	1.37	1.16	1.40	1.42	1.09	1.90
	Fourth	2.20	1.95	2.40	2.02	1.98	1.94	1.76
	Fifth	1.85	1.92	1.12	2.10	2.47	1.54	2.03
Khr-v	First	1.76	1.76	1.96	2.10	1.86	1.94	1.75
	Second	1.81	1.85	1.87	2.02	2.14	1.67	1.61
	Third	1.04	1.56	1.39	2.03	2.44	1.63	1.96
	Fourth	2.43	2.82	2.34	2.40	2.06	2.00	1.96
	Fifth	2.35	2.12	2.28	2.29	2.43	1.92	1.81
Average for the two test subjects (M ± m)		1.82± 0.13	1.89± 0.12	184 ± 0.13	1.99± 0.09	2.02 ± 0.1	1.71± 0.08	1.75± 0.09

Data for the excretion of ammonia in the urine are of definite interest as an index of shifts which tend to support the acid-alkaline balance in the organism during a change of gas composition of the inhaled air (Table 5). /103

TABLE 5. EXCRETION OF AMMONIA IN THE URINE (IN GRAMS) FOR TEST SUBJECT BY DAYS OF THE EXPERIMENT DEPENDING ON THE GAS ENVIRONMENT.

Test subject	Gas composition by cycle	Days of cycle						
		1	2	3	4	5	6	7
F-v	First	0.67	0.69	0.61	1.90	0.81	1.02	1.04
	Second	1.02	0.71	1.84	0.72	0.98	0.73	0.74
	Third	0.84	2.0	0.91	1.02	1.17	0.92	1.46
	Fourth	0.70	0.70	2.33	0.90	1.85	0.98	1.18
	Fifth	1.24	1.38	1.11	1.27	1.79	1.21	1.47
Khr-v	First	0.52	0.63	0.55	0.96	1.11	1.04	1.05
	Second	1.01	1.21	0.78	0.81	1.15	0.93	0.97
	Third	0.99	1.11	1.09	1.55	1.25	1.06	0.77
	Fourth	1.03	1.36	0.97	0.87	0.68	1.97	1.65
	Fifth	1.46	1.86	1.18	1.67	1.97	1.45	1.31
Average for the two test subjects (M ± m)		0.96 ± 0.08	1.16± 0.14	1.14± 0.17	1.17± 0.11	1.28± 0.14	1.13± 0.1	1.16 ± 0.08

It is an interesting fact that a small amount of ammonia in the urine of the test subjects was noticed only on the first three days of the experiment.

Beginning on the fourth day, when an excess of carbon dioxide appeared in the atmosphere of the chamber, the elimination of ammonia in the urine increased and remained basically at an increased level for all of the subsequent days and was not related to the change of parameters of the gas environment.

According to data in the literature pathological changes on the part of the internal organs including the gastro-intestinal tract (Mogendovich, 1961) can be observed during prolonged hypokinesia. There was a practical interest in tracing the functional condition of the gastro-intestinal tract by the integrative index: assimilation of proteins from the ration. Data of the metabolic tests conducted during the third cycle can be seen in Table 6.

TABLE 6. ASSIMILATION OF PROTEIN BY THE TEST SUBJECTS DURING THE EXPERIMENT.

Test subj.	Taken daily, g	Eliminated with feces for a day, g	Assimilation	
			g	%
F-v	112	14.25	97.75	87.5
Khr-v	114	15	99	86.7

Assimilation of food protein within limits of 86-87% is considered high. In addition, it indicates a good functional condition of the gastro-intestinal tract during the experiment.

The change in the food value of the ration in favor of a reduction of the caloric-cally active components as well as an increase in excretion of total nitrogen in the urine definitely must reflect the condition of the water metabolism as a whole and its structure. Under such conditions, during an absence of intensive physical stress, there is normally no substantial tension of water metabolism and low development of dehydration of an organism. But the presence of the above factors, the definite hypokinesia and the changed gas environment might lead to a change of the water metabolism.

Table 7 presents data on the condition of the water-salt metabolism for the test subjects on the first days of the experiment before the onset of the action of changing gas environment.

From the data in Table 7 it follows that the water losses outside the kidneys decreased noticeably along with a transition to light under conditions of limited movement for both test subjects, evidently as a result of more comfortable conditions for heat exchange and the decrease of heat production. At the same time the amount of daily urine increased, which might be explained practically by the slightly changed quantity of water and food intake. There was a simultaneous increase of electrolytes in the urine. /105

TABLE 7. CONDITION OF THE WATER-SALT METABOLISM FOR THE TEST SUBJECTS AT THE BEGINNING OF THE EXPERIMENT.*

Period of observation	Body wt. in morning on empty stomach, g	Qty. of urine, ml	Water losses outside kidneys, g	Daily elimination of urine, mg					Wt., g	
				Na	K	Cl	Ca	P	Food	Water
Test subject F-v										
On the eve of the experiment.	82550	830	1970	1825	830	2430	66,5	1162	1025	1425
1st day	82200	1380	1110	3170	2341	5725	345	2070	990,5	1200
2nd day	81900	1360	1024	4080	2445	5150	202	2590	1034	1450
Test subject Khr-v										
On the eve of the experiment.	64850	1340	1907	2280	1740	4500	107	1745	1047	1400
1st day	64350	1650	751	2810	1650	5540	173	2310	1001,5	1300
2nd day	64250	1570	784	2830	2350	7180	550	2200	1043	1400

*Translator note: Commas indicate decimal points.

Subsequently the action of the changed gas environment took place against the background of the changes noticed. It seemed reasonable to compile data on the condition of the external water metabolism in conformity with the influencing factors in each of the seven-day cycles. Consideration of the effect of the

hypoxic and hypercapnic gas mixture on the amount of extrarenal water loss was of special interest to us. Under conditions of comfortable temperature and during the absence of the action of substantial physical stresses extrarenal water losses are determined chiefly by the value of the imperceptible water losses, strictly due to perspiration losses and insignificant sweating. Water losses due to perspiration include water which evaporates from the surface of the respiratory tracts and the skin. Therefore, under conditions of changing gas environment and increase of pulmonary ventilation an increase of perspiration water losses in the lungs and respiratory tracts and the corresponding increase of the total extrarenal water losses could be expected. Table 8 presents data on the value of the extrarenal water losses in each of the seven-day periods in accordance with the change of gas environment.

TABLE 8. EXTRARENAL WATER LOSSES FOR THE TEST SUBJECTS UNDER CONDITIONS OF NORMAL AND CHANGED GAS ENVIRONMENT (in mℓ).

Test Subject	Period	Days						
		1st	2nd	3rd	4th	5th	6th	7th
F-v	1st	1110	1024	1417	1032	1501	898	829
	2nd	766	1360	964	1007	604	714	609
	3rd	1237	1780	1734	1219	1298	1055	859
	4th	1125	1247	1555	1184	910	1775	1259
Khr-v	1st	751	784	1237	575	1485	738	985
	2nd	684	990	1109	978	1065	1236	839
	3rd	1299	1085	1082	1308	1658	—	1474
	4th	766	1088	1799	1157	1220	1415	1417
$M \pm m$		967±94	1169±67	1361± ±118	1057±85	1217± ±158	1118± ±142	1033± ±118

The data obtained show that in half of the cases there is an actual increase of extrarenal water losses during the transition to a hypoxic mixture. Upon subsequent enrichment of the gas environment with CO_2, on the contrary, /106 there is a reduction of extrarenal water losses for the majority of cases. A return to the normal gas composition for test subject Khr-v was again accompanied by an increase of extrarenal water losses, but this happened in only half of the cases for F-v.

Data on the excretion of sodium, potassium, chlorides, calcium and phosphorus in the urine are presented in Tables 8, 9, 10, 11, 12, and 13. The excretion of sodium, potassium, chlorine, calcium and phosphorus in the urine during the experiment remained within the limits of normal values and was determined by the food composition of the ration. We detected no noticeable effect /107 of the cyclical changes of the gas environment on the excretion of these macroelements in the urine.

TABLE 9. EXCRETION OF SODIUM IN THE URINE OF THE TEST SUBJECTS IN THE EXPERIMENT (in mg/day).

Test Subject	Period	Days						
		1st	2nd	3rd	4th	5th	6th	7th
F-v	1st	1825	3170	4080	2520	1798	2180	1185
	2nd	2280	2810	2830	2780	1860	1910	1530
	3rd	1935	1440	2970	2290	2070	1870	1680
	4th	2300	2580	3990	5760	2290	2050	—
Khr-v	1st	—	—	2080	4040	1850	1750	—
	2nd	—	—	5570	2680	1880	2390	—
	3rd	—	—	2000	1871	—	—	—
	4th	—	—	1850	2640	—	—	—
$M \pm m$		2085±120	2500±371	3171±65	3072±81	1958± ±154	2025± ±514	1465± ±121

TABLE 10. EXCRETION OF POTASSIUM IN THE URINE OF TEST SUBJECTS DURING THE EXPERIMENT (in mg/day).

Test Subject	Period	Days						
		1st	2nd	3rd	4th	5th	6th	7th
F-v	1st	830	2341	2445	1472	1258	1800	711
	2nd	1730	1650	2350	1750	1330	1130	1020
	3rd	1182	935	742	1231	1675	1530	1120
	4th	1150	1105	748	3750	1387	865	—
Khr-v	1st	—	—	1040	1681	970	1010	—
	2nd	—	—	2560	824	1130	1455	—
	3rd	—	—	640	1001	—	—	—
	4th	—	—	840	1530	—	—	—
$M \pm m$		1223±186	1507±315	1420±85	1654± ±125	1291± ±352	1300± ±370	930±124

TABLE 11. EXCRETION OF CHLORIDES IN THE URINE OF TEST SUBJECTS IN THE EXPERIMENT (in mg/day).

Test Subject	Period	Days						
		1st	2nd	3rd	4th	5th	6th	7th
F-v	1st	2430	5725	5150	5280	3370	3290	4240
	2nd	4500	5540	7180	4910	5520	4560	6220
	3rd	4470	3355	4390	4360	3690	3520	3320
	4th	6170	6090	4670	4840	4210	3620	—
Khr-v	1st	—	—	3070	4840	4000	3820	—
	2nd	—	—	5270	4570	3900	3080	—
	3rd	—	—	3320	3560	—	—	—
	4th	—	—	3483	3530	—	—	—
$M \pm m$		4392±759	5177±463	3199±154	4486± ±181	4115± ±778	3655± ±260	4593± ±850

TABLE 12. EXCRETION OF CALCIUM IN THE URINE OF TEST SUBJECTS IN THE EXPERIMENT (in mg/day).

Test Subject	Period	Days						
		1st	2nd	3rd	4th	5th	6th	7th
F-v	1st	66,5	345	202	147	306	256	122
	2nd	107	173	550	187	731	218	225
	3rd	257	104	170	75	143	148	60
	4th	161	258	248	202	138	108	—
Khr-v	1st	—	—	197	265	—	253	—
	2nd	—	—	244	135	—	207	—
	3rd	—	—	172	94	—	—	—
	4th	—	—	59	144	—	—	—
$M \pm m$		148±50	220±52	230±98	156±20	329±71	198±24,8	135±48

On the basis of the data obtained we can conclude that a tendency towards reduction of the negative nitrogen balance during hypokinesia is noticed during the action of an active gas environment with oxygen deficit and excess of carbon dioxide which increases the muscular activity of the respiratory muscles.

TABLE 13. EXCRETION OF PHOSPHORUS IN THE URINE OF TEST SUBJECTS IN THE EXPERIMENT (in mg/day).

Test Subject	Period	Days						
		1st	2nd	3rd	4th	5th	6th	7th
F-v	1st	1162	2070	2590	1890	1975	1520	1580
	2nd	1745	2310	2200	2700	2930	1530	1690
	3rd	1615	1050	1700	1625	1890	1950	880
	4th	1840	1230	2740	2600	2780	920	—
Khr-v	1st	—	—	1040	840	960	1010	—
	2nd	—	—	1160	1405	1125	—	—
	3rd	—	—	1760	793	—	—	—
	4th	—	—	885	895	—	—	—
$M \pm m$		1590±41	1665± ±180	1759± ±286	1593± ±149	1943±287	1386±333	1383±252

The peculiarities observed in nitrogen metabolism under the effect of an active gas environment require further study. Evidently, the hypoxic gas environment can increase the extrarenal water losses which must be considered when standardizing the consumption of water for similar conditions. /108

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CONDITIONS OF ASCERTAINING AND CONDITIONING MAN'S CAPACITY TO DISTINGUISH THE COMPOSITION OF A BREATHING ATMOSPHERE.

I. S. Breslav, A. G. Zhironkin and A. M. Shmeleva

ABSTRACT: The problem of the capability of man to distinguish changes in the composition of inhaled air is of great practical interest but has so far been very little studied.

This work presents results of investigations which show that this capability can be developed by the method of active selection of preferred gas mixtures on the part of man, and also by the gradual reduction in the amount of oxygen in the breathing air. Man's ability to distinguish changes in the composition of gas mixtures improves under conditions of a constant (fixed) level of pulmonary ventilation.

An individual variability of reactions in different people to hypoxic and hypercapnic environments was noted. During repeated exposure to different respiratory mixtures man can more clearly differentiate between these mixtures according to his sensations.

Thus, the ability of man to distinguish definite changes in the amount of separate components of breathed air according to his sensations may be imporved. It is possible that such conditioning would be valuable in providing security during high-altitude and space flights.

Obviously, artificial respiratory mixtures of varying composition are going to be used under conditions of spaceflight and landing by man on other heavenly bodies. One of the problems arising in this connection is the capacity of man to distinguish changes in the amount of oxygen and carbon dioxide in the gas medium. /109

It is assumed that man is far from always able to notice by his own sensations even a threatening drop of partial pressure of oxygen in the inhaled air. Much has been written concerning this by physiologists (Holden, Priestley, 1937), and by specialists in aviation medicine (Armstrong, 1954).

It is true that a number of "subjective" symptoms of hypoxic condition in man are known, as well as complaints which accompany an increase in the amount of carbon dioxide in the air. However, the capability of man to evaluate the physiological sufficiency of the gas medium on the basis of such sensations so far has not been subjected to sufficiently serious experimental verification.

We were able to show that such a capability can be developed by the method of active selection on the part of man of preferable respiratory mixtures.

In the experiment the test subject alternately breathed two different mixtures through a mask: an inert one (usually this was normal air) and an active one (hypoxic or hypercapnic). The test subject did not know the composition and order of alternation of these mixtures. We began the test with breathing from each of the mixtures for five, ten or fifteen minutes (assigned respiration), and then for ten-fifteen minutes the person was able to select any one of these mixtures, being influenced by his own sensations (free choice). The test subject switched from one gas mixture to another with the aid of a pushbutton switch. Discrimination of one medium or another was evaluated by the index of selecting the inert mixture (S_t) — by the variation between periods of time during which the test subject breathed the inert (t_i) and the active (t_d) mixtures, attributed to the duration of the period of free choice (T):

$$S_t = \frac{t_d - t_i}{T}.$$

After the test the test subject explained in a written report his sensations while breathing the mixtures and his motives for selection. The experiments were conducted on sixty-four healthy persons of both sexes from eighteen to thirty years of age. /110

The studies carried out by this method showed that man can with certainty distinguish mixtures which differ from normal air when the amount of oxygen is reduced by 10.5% and less (Breslav, Salatsinskaya, 1967-1968), or if the concentration of carbon dioxide exceeds 2.5-3%. Even breathing such mixtures for five minutes made the majority of test subjects want to avoid them. This is shown by the preference for the inert mixture, that is, for air (negative index for selection of active medium), while simultaneously complaints of difficulty

in breathing and disturbance of general well-being during inhalation of the corresponding mixtures arise.

Physiological analysis of the experimental data shows that hypoxic and hypercapnic shifts which develop in the organism are the basis for negative reaction of man to these mixtures, and the increase in consumption of energy due to the increased pulmonary ventilation is partially responsible for this negative reaction.

What has been said is confirmed by the following regularity: by lengthening the period for assigned respiration of each of these mixtures to ten-fifteen minutes, when a definite deficit of oxygen or excess of CO_2 has managed to develop in the organism, a man might distinguish weaker degrees as hypoxia (15% O_2) as well as hypercapnia (all told 1-2% CO_2). Thus, within certain limits, the longer a person breathes one mixture or the other the easier it is for him to distinguish it from air. The following data illustrates the role of the time factor in man's ability to distinguish hypoxic media:

Amount of O_2 in respiratory mixtures, %	15	12	10.5
Average decrease in oxygenation of blood for the fifteen minute period of inhalation, %	5	14	20
Period of assigned respiration, in which the negative reaction becomes significant, min.	15	10	5

Man is capable of distinguishing not only a transition from one mixture to another but also a gradual change of composition in the respiratory medium. The following experiment proves this.

The test subject breathed through a mouthpiece in a closed rebreathing system, which was filled with air. As the oxygen was consumed the amount of it in the air of the system gradually fell (the separable CO_2 was removed by an absorbent). When the test subject noticed "a change in composition of the respiratory mixture" (words of his instructions), he gave a signal. Then rebreathing continued until onset of the express phenomena of oxygen deficiency (tremor, disturbance of muscular tonus, loss of consciousness) or until the test subject realized he was unable to breathe any longer. In the latter instance he switched /111
over to the air by himself.

Usually a person noticed a change in his well-being (difficulty in breathing, lack of air, infrequently--pounding in his temples, dizziness) by the eighth or ninth minute of rebreathing in this system, when it had an average amount of oxygen of about 10% and when the oxygenation of the blood was approximately 80%. Following this the test subject was able to breathe for two-three more minutes in the closed system, until the test was halted because of the indicated circumstances. The amount of O_2 in this system by the end of the test fell to 6%, and oxygenation of the blood of the test subject fell to 60% or somewhat lower (Figure 1).

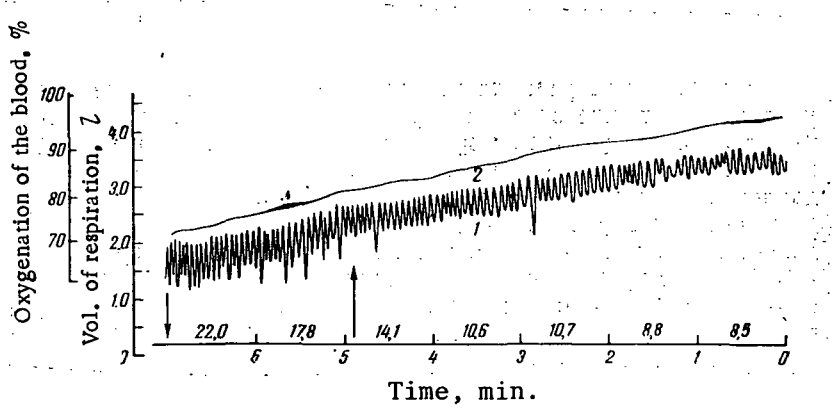

Figure 1. Spirogram (1) and Oxygen Saturation of the Blood (2) of One of the Test Subjects While Breathing Under Conditions of Increasing Hypoxia (Read From Right to Left). Digits underneath the spirogram — minute volume of respiration (in l). Arrow pointing upwards, moment when the test subject gives a signal indicating change of his well-being; Arrow pointing downward, moment of active switching to respiration of external air (end of test).

The calculation shows that under these conditions a man has sensations which lend themselves to verbal recording, approximately with the same deficit of oxygen in the organism as during respiration by "threshold" (in the sense of their discrimination from air) hypoxic mixtures.

Human discrimination of a changed composition of respiratory mixtures became much less keen when the respiration took place with a constant level of ventilation. This was achieved by setting respiratory movements at a definite

rhythm and depth. The ventilator reactions in such a fixed schedule were restrained by the test subject himself. Under these conditions human thresholds for negative reactions to hypoxic and hypocapnic media seemed noticeably lower than during free respiration. Judging from our observations, the cause of the given phenomena is contained in the conflict between intensive chemoreceptive stimulation and cortical pulsations, which delay the reflex response to this stimulation.

The negative reaction of man to hypoxic mixtures was also reinforced during /112
a stay in the mountains, at an altitude of two-three thousand meters above sea level, which is evidently related to the general hypoxemic "background" which was conditioned by the reduction of partial pressure of oxygen in the medium (Breslav, 1968).

The capability of distinguishing respiratory mixtures was quite variable in different persons. Thus, subjects G. I-v and V. P-v, only after five minutes of inhalation, distinguished without difficulty the air and mixtures of comparatively slightly changed composition (15% O_2 in nitrogen or 2% CO_2 in air), in ratios at which the majority of people detected no regular reaction. These persons served as a standard of the capability of man to discern changes of composition of the respiratory medium. On the other hand, there were also cases (although rare) when the test subjects selected mixtures which caused sharp hypoxic or hypercapnic shifts in them, for example, mixtures of 7% O_2 or 5% CO_2.

It turned out that hypoxic mixtures are easily distinguished by persons with more regular and relatively slow and deep respiration.

To mixtures with 12-10.5% amount of oxygen they reacted with a noticeable increase in pulmonary ventilation while test subjects who poorly distinguished hypoxic media still had not had any ventilator reaction to these mixtures. More clearly defined hypoxic mixtures (9-7% CO_2) caused a greater increase of minute volume of respiration (MVR) in the "good discriminators" than in the "poor discriminators." It seemed that the respiratory reaction in persons who readily react to hypoxic mixtures is more beneficial for compensation of the oxygen deficit. However, a drop in oxygenation of the blood due to the effect of these mixtures in the "good discriminators" was not less but even somewhat

higher than in the "poor discriminators." This combination of expressed ventilator reaction with relatively pronounced hypoxemia, and consequently, with more intensive chemoreceptive pulsation, is probably one of the causes of some "sensitivity" of certain persons to a decrease in the amount of oxygen in the respiratory medium.

The majority of those who easily distinguished an addition of carbon dioxide had relatively rare respiration with a small MVR. Their ventilation reaction to hypercapnia was expressed to approximately the same degree as in the remaining subjects.

There is probably something in common in the capacity of man to distinguish hypoxic and hypercapnic media. Three of the subjects (G. I-v, V. P-v and T. Ch-va) were among those who keenly distinguished mixtures either with reduced amount of oxygen or with increased concentration of carbon dioxide. V. M-v likewise reacted equally weakly to both circumstances. It is true that such a /113
parallelism is not the rule: test subject N. L-va easily distinguished the hypercapnic mixtures but distinguished the hypoxic ones poorly.

The peculiarities of certain parameters of respiration noted above still do not explain why one group of persons easily differentiates change in the composition of the gas medium and another group does it poorly. We discovered no regular discrepancies between the above groups for such indices as sex, age (it is true that all of the test subjects belonged to similar age groups) and according to anthropometric data including vital capacity of the lungs.

It might be thought that dissimilar reactions of different persons to definite gas mixtures relate not only to individual peculiarities in the regulation of respiration but also to certain properties of their higher nervous system in the sense of a greater or lesser sensitivity to signals from the external environment (Sechenov's "doubt feelings"). In studies by A. B. Gandel'sman et al. (1966), they noted that tran trained athletes have an especially keen capacity to differentiate hypoxemic shifts in their organism. We are inclined to support such a regularity: all of the test subjects who distinguished hypoxic mixtures better than the others systematically engaged in athletics but in the group of those who distinguished such mixtures poorly (five persons) only one person took part in sports.

But how can we relate this to the fact that, according to our data, the persons who easily differentiate hypoxic respiratory media suffer a more significant drop in saturation of the blood with oxygen? Evidently the high resistance to oxygen deficiency is not at all necessarily connected with the maintenance of an especially constant level of oxygenation of the blood. "It is expedient," Tribukayt (1963) wrote, "to view the amount of oxygen in the blood as a component for providing tissues with oxygen, but not in the sense of a homeostatically regulated assigned value." Actually, when speaking about the adaptation to hypoxia (whether it be respiration in an hypoxic medium or heavy labor), the matter is above all one of economy of utilization of oxygen. This fact was recently demonstrated in the study by A. A. Artynyuk (1968). It was revealed that for highly trained athletes the drop in oxygen saturation of the blood during muscle stress is even greater than for unconditioned persons. It is possible that the increased hypoxemic shift itself enables a person to more easily "feel" the lack of oxygen and adapt to it more quickly. Herein derives the hypothesis that the ability of a person to keenly sense shifts in the internal environment during respiration of hypoxic mixtures might be one of the criteria of his good capacity to adapt to an oxygen deficit.

Why not directly train this capacity for distinquishing changes in the composition of the respiratory medium? Evidently it is possible. /114

After repeated experience with hypoxic mixtures man's discrimination of them becomes keener. Thus, in one of the groups, which consisted of twelve test subjects who had never encountered hypoxic media, only nine persons had a negative reaction to the mixture with 9% O_2. In the other group who had previously taken part in such tests, the same mixture caused a negative reaction in all twelve test subjects.

Another example is two consecutively conducted tests with rebreathing in a closed system (i.e., with gradually increasing hypoxia). The methodology of these tests was described above. In the first test the majority of the test subjects, during rebreathing, not only could not notice the sensations which are related to a lack of oxygen but also were unable to switch to the atmospheric air until they lost consciousness. In the second test the same test subjects detected the moment of onset of significant hypoxemia, and the majority actively

switched to the air at the end of the test, until they had the capacity for further respiration in the hypoxic medium. Here we present data on the effect of conditioning on the capacity of man to distinguish a gradual decrease in the amount of oxygen in the medium during respiration in a closed system.

Test subjects	Exposure	
	First	Second
Who distinguished changes of composition of the medium	5	11
Who did not distinguish a change in the composition of the medium	6	0
Who actively switched to the air	4	7
Who were switched by the person running the experiment due to signs of acute hypoxia	7	4

Evidently in this case there is an improvement in analysis of increasing chemoreceptive signalization by the higher levels of the central nervous system (Gandel'sman et al., 1966).

In order to determine if it is possible to condition the capacity of man to distinguish the addition of carbon dioxide in a respiratory medium, we repeatedly exposed a group of six persons to the same pair of gas mixtures: the inert one was air, and the active one was 2% CO_2 with the usual amount of oxygen. The period of assigned respiration of each mixture was five minutes.

In the first presentation only one-half of the test subjects showed a negative reaction to the hypercapnic mixture, and even less during the second. Later the test subjects were given the task of training themselves to distinguish the active mixture from the inert. At the end of each test they were told whether they had correctly determined which of the buttons connected the hypercapnic mixture and which one connected them with the air (the relation of buttons to mixtures changed in a random order). Such information enabled the majority of test subjects to correctly distinguish the mixtures beginning with the second or third test (Figure 2). /115

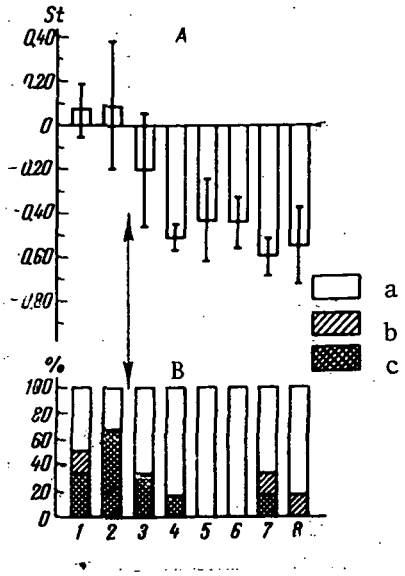

Figure 2. Dynamics of Human Discrimination of a Mixture Containing 2% CO_2 During Repeated Presentations of This Mixture. A, Index of selection of inert mixture (St); B, Ratio,(in %) of the number of test subjects, who identified the mixtures correctly (a), incorrectly (c) and who did not distinguish the mixtures (b). The arrow indicates the moment of correct identification of the gas mixture by the test subjects.

The reason for the errors which we observed during discrimination of the gas media are interesting. These errors were not only in the incorrect identification of the mixtures but also in the distorted respiratory reaction. For example, test subject E. K-va showed a normal increase of ventilation during respiration of the 2% CO_2 in the first test. In the fourth test she mistook the air for the inert mixture and therefore showed preference for the hypercapnic mixture. Breathing the air in this test caused an abrupt hyperventilation, and breathing the mixture with 2% CO_2 on the contrary caused hypoventilation while a change of respiration during the conversion of mixtures was instantaneous when the composition of the new mixture was of course not yet able to have any effect (Figure 3). Here there is a suggested reaction to the respiratory medium: understanding the physiology of respiration the test subject followed her own (in this instance an incorrect one) evaluation of the mixtures and unintentionally produced in an exaggerated form, the ventilation reaction to this mixture which she thought was hypercapnic.

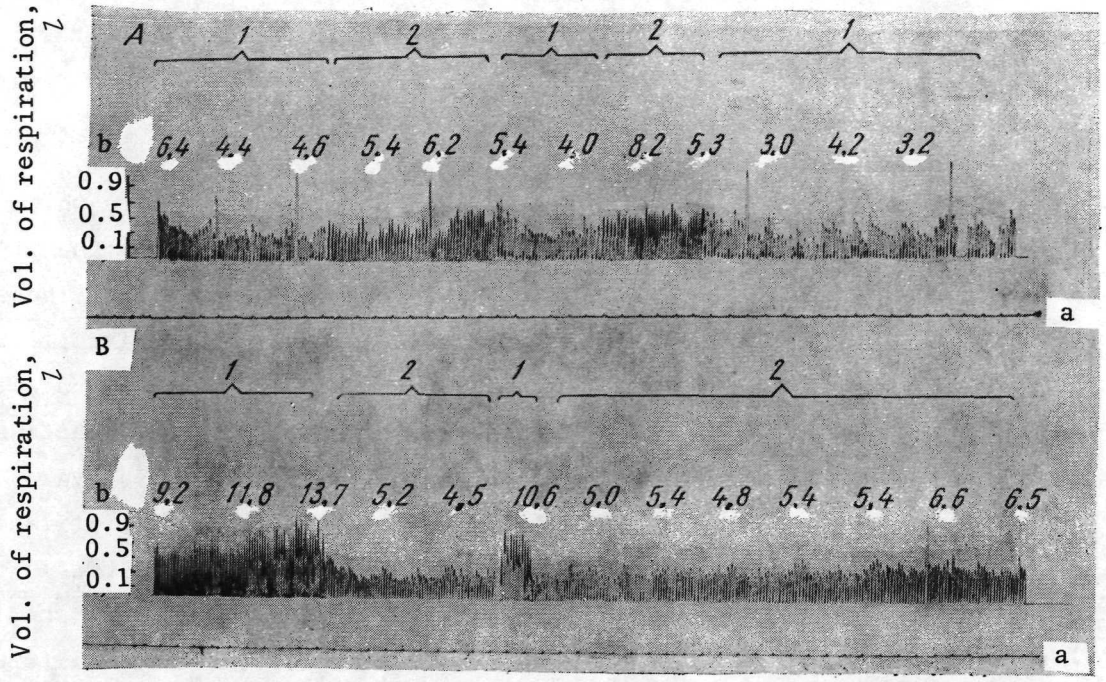

Figure 3. Spirogram of Test Subject E. K-va During Respiration of Air (1) and a Mixture Containing 2% CO_2 (2). Correct (A) and mistaken (B) identification of mixtures by the test subjects. a, Time, 30/5 sec; b, Respiratory minute volume, in l.

Incidentally V. I. Voytkevich (1952) observed a sufficient conditioned--reflex reaction — abrupt increase of ventilation in response to a verbal signal of a forthcoming respiration of hypoxic mixture (the command: "I'm giving you a harmful mixture!") in his test subjects. In the investigations mentioned above it was shown that if a person is instructed on the signs of hypoxia and hypercapnia before the test he can more accurately evaluate shifts in the gas regulation during the corresponding actions.

Evidently, vocal signalizing is of great importance in the process of conditioning the capacity of man to distinguish respiratory media. As we saw, individual peculiarities of the persons in this connection are quite varied. Examples which have been described in this work naturally cannot yet serve as a ready basis for developing methods of conditioning man to discriminate gas mixtures. But in principle, improvement of this capacity is completely possible. /117

It is sufficient to indicate the problems in providing protection of pilots and cosmonauts from oxygen deficiency. Armstrong, in his famous book, (1954), paid particular attention to the harm brought about by an indifferent attitude of aviation specialists and pilots themselves to the dangers related to altitude hypoxia and to the necessity of using oxygen at specific altitudes. Armstrong thought that the most important reason for such an indifference was the lack of adequately characteristic sensations for hypoxia. "For almost all of the disorders which occur in an organism nature gives warning signals in the form of pain and other unpleasant sensations...for altitude disease, on the contrary, not only are symptoms lacking, but sometimes a good feeling of well--being is completely maintained...therefore, it is unfortunate," argues Armstrong, "that altitude sickness is not accompanied by pains and other unpleasant sensations. If this was the case then both the health of the flight crew and the safety of pilots would be ensured."

To these words we may now add that we are able to detect and reinforce under definite conditions insufficient "subjective" signals during hypoxia. Foreign literature contains recommendations for creating the ability to "sense" ionizing radiation, in order to provide a natural system for notification of danger [10]. We propose that it is even easier (and sometimes possibly even more important) to improve the capacity of a man to sense changes in the composition of the respiratory medium which may be critical for his work, health and even life.

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EFFECT OF HYPEROXIC MEDIUM ON CELLS, TISSUES AND ORGANS OF EXPERIMENTAL ANIMALS

Ye. F. Kotovskiy and L. L. Shimkevich

ABSTRACT. This article is a review of the authors' and literature data on the general morphology, ultrastructure and histochemistry of various organs of experimental animals during hyperoxia. Basically, information was presented on the effect of 100% O_2 at normal barometric pressure. Oxygen damages the vascular wall, eosinophilic infiltration of tissues, and has a pronounced action on the condition of the ultrastructure and metabolism of cells. Initially there is activation of metabolism of cells without visible morphological changes. This period (6 hours) is considered on the whole as harmless for the organism. Later (after 12 hours) phenomena of a pathological nature arise, including obesity of cells, decrease in the amount of RNA and protein, disorders of the ultrastructure of mitochondria, and suppression of oxidizing enzymes. A compensatory increase in the activity of glycolytic enzymes ensues following the appearance of secondary hypoxia of tissues. The general types of cell reactions are discussed as well as the specific peculiarities of the reaction of different tissues and organs.

Numerous studies carried out in recent years have significantly expanded /118
our concepts of the nature of the effect on the animal organism of high concentrations of oxygen in the surrounding atmosphere. In spite of the fact that a relatively small number of reports have been devoted to the study of the morphological substrate of oxygen infections, our knowledge of this area is gradually becoming more definite and less contradictory. Regarding the action of oxygen on metabolism, since this problem is so immense, the information which we do have explains the condition of only part of the components of the general metabolic chain. Therefore the combination of methods of general morphology, electron microscopy and cytochemistry, as in various other investigations, can help us in interpreting the observed facts.

The opinion that oxygen has various effects on an organism depending on the value of the barometric pressure of the inhaled gas mixture, its concentration of oxygen and duration of exposure time is now generally accepted. According to physiological and biochemical data, an increase in pressure increases the reaction of the CNS until oxygen cramps appear (Gershenovich, Bronovitskaya, 1955; Zhironkin et al., 1965). However, because studies of the gas mixtures at normal and even at reduced barometric pressure have the greatest interest for development of aviation and space medicine, in our experiments we emphasized the corresponding conditions of oxygenation.

The high amount of oxygen in the surrounding atmosphere has a significant effect on the entire organism, but at normal barometric pressure changes arise which are related, first of all, to damage to the system of respiratory organs. The initial signs, as a rule, are irritation of the upper respiratory pathways accompanied by coughing, hyperemia of the mucus membranes, painful sensations in the throat and behind the chest (Popov, 1941; Uzarin et al., 1966; Comroe et al., 1945). Unfortunately detailed morphological investigations of the condition of the mucus membranes of the ear and nose have not been conducted. In our experiments we observed increase in secretion of mucus by the goblet cells of the bronchi, and in more serious cases we observed destruction of the flickering cilia of the epithelium. If the animals are kept in an atmosphere /119
of pure oxygen at 1 absolute atmosphere for 12 hours peribronchial edema and eosinophilic infiltration of the surrounding tissues occur. During toxic exposure (six absolute atmospheres for 30-60 minutes) similar phenomena set in practically immediately following decompression and are maintained throughout the following two days.

Pathological changes in the lungs during prolonged exposures to oxygen are characterized by increasing phenomena of plethora, edema and atelectases. During the action of 100% oxygen at normal atmospheric pressure disorders of the structure of the pulmonary parenchyma occur rather quickly and become noticeable after three to six hours of oxygen respiration. It is true that these changes initially are reversible, because they disappear soon after

transferring the animals into an atmosphere of air. When the exposure times are longer, signs of toxic effects of oxygen on different structures of the lungs gradually begin to appear.

According to modern concepts, the surfaces of the alveoli, which are adjacent to the air, are covered by a layer of insoluble lipoprotein, with a thickness of about 50 Å, under which a jelly-like lipoprotein layer with a thickness of 500 to 2,000 Å is located. The substance of the second layer may be dispersed in a physiological solution and impart the capacity of reduction of surface tension to the pulmonary extract. This surface-active substance (surfactant) is considered an important factor of mechanical stabilization of the alveoli (Pattle, 1967). It is supposed that oxidation of the lipid fraction of the surfactant takes place during an excess of oxygen, which leads secondarily to dystelectases (Webb et al., 1966). In an ordinary histological investigation numerous atelectases which alternate with emphysematous sections are usual in oxygen damage to the lungs.

In the opinion of Kistler et al., (1967), primary cellular damage is localized in the endothelial cells of the pulmonary capillaries. The authors noted phenomena in them of condensation of cytoplasm, injuries to the organelles and even complete fragmentation of cells. They also observed swelling and vacuolization of the endothelial cells, which led to embolism of the capillaries and capillary stasis (Nassery et al., 1967). But injuries of the epithelial layer are often sparser. However, during the prolonged action of oxygen at one absolute atmosphere (24-72 hours) or at increased pressure (3-6 absolute atmospheres) phenomena of intracellular edema, destruction of the mitochondria with formation of myelin figures, reduction in activity of oxidation enzymes (especially enzymes of the Krebs cycle and cytochromic system), and an increase /120 in the quantity of lipids (Figure 1) are observed.

The cellular composition of the alveolar septulum changes (Kistler et al., 1967) and there is an increase in the number of granular pneumocytes and macrophages as well as the septal cells of the capillaries. Edematous fluid accumulates

in the interstitial spaces, and contains fibrin and leucocytes, leading to expansion of the interalveolar septulum and thickening of the air-blood barrier from the normal 1.5 μ to 2-3 μ and more. As a result the gas exchange is retarded which, in the opinion of some authors, has an accommodative nature (Schaffner et al., 1967).

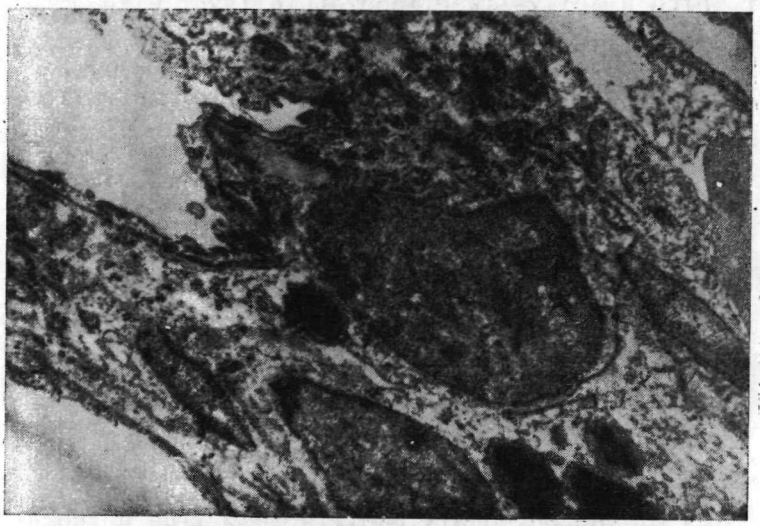

Figure 1. Electron Photomicrograph of the Lung of a White Rat. Pure oxygen at 6 ata. Accumulation of lipid granules in the cytoplasm of the cells. 8,000 ×

Under the continuous action of oxygen in the lungs crude pathomorphological changes are created: acute perivascular and peribronchial edema, hemorrhaging and pneumonic foci. As a rule, animals die with symptoms of acute pulmonary edema.

The morphophysiological and pathological processes which arise in an organism during the action of oxygen naturally are not limited only to the lungs. Study of the nervous system is of great interest. It is true that according to the data in the literature (Zhironkin et al., 1965), the problem of the study of the effect of oxygen at normal barometric pressure /121 on the function of the central nervous system has received little attention. To this we must add that the number of morphological observations has been extremely limited.

Generalizing from the literature and their own data, A. G. Zhironkin, A. F. Panin and P. A. Sorokin (1965) came to the conclusion that oxygen has an inhibiting effect on the function of the central nervous system during exposure

times which do not exceed 4-5 hours under conditions of normal atmospheric pressure (1 ata). It is true that there are also observations which confirm the increase of the psychic activity in persons during oxygen respiration, even leading to euphoric conditions. In our study of the short-term action of oxygen (3-6 hours) on white rats we observed a reduction of the activity of lactic acid dehydrogenase in a majority of structures of the brain. Regarding the activity of aerobic processes (appearance of succinate dehydrogenase and cytochromoxydase), their intensity changed dissimilarly in different sections of the central nervous system and even in different cellular elements.

Thus, for example, in the cortex of the cerebellum the activity of succinic acid dehydrogenase was reduced in cells of the molecular and granular layers and was increased in the ganglionic cells; in the cerebral cortex the enzymatic activity was somewhat reduced at first and then increased. Similar results were attained in a tissue culture in vitro (Uzorin et al., 1966). Saturation of a nutrient medium with a gas mixture containing 95% oxygen during cultivation of Purkinje cells of the cerebellum and spinal ganglia of rabbits led to increase of the succinate dehydrogenase activity.

Here it is necessary to note that there is no significant deviation during the oxidation processes in the brain during short-term exposures to oxygen. The morphological observations indicate the absence of some noticeable structural changes.

The longer action of oxygen on animals (12-24 hours) led to an increase in succinate dehydrogenase activity in practically all of the elements of the nervous system and an increase in the activity of lactic acid dehydrogenase principally in the glial cells. Simultaneously in different sections of the brain (cerebral cortex, subcortical formations, cerebellum) there appeared changes of a pathological nature: perivascular edema, swelling of the nerve fibers and dystrophic phenomena in nerve cells of a nonspecific nature (chromatolysis of the nuclei, reduction of the amount of chromatophilic substance in the neuroplasm), which were more pronounced in the subcortical sections. Electron microscopy confirms the presence of intercellular and intracellular edema, expansion of the synaptic fissures, and an increase in /122
the number of synaptic vesicles.

During prolonged breathing of pure oxygen at 1 ata (more than 48 hours) and during the action of high oxygen pressures (6 ata for 30-60 minutes) significant destructive changes of the mitochondria are detected (swelling, reduction of the number of cristae), and during the investigation of the mitochondrial enzyme, succinate dehydrogenase, a reduction in its activity is noticed. Simultaneously there is frequently a compensatory increase in the activity of the glycolytic enzymes — lactic acid dehydrogenase.

It is interesting to note that dense mitochondria which contain a large number of cristae are encountered in nerve and glial cells in addition to the above-described changes. Clear pictures of alteration of the mitochondria are also very frequently observed (significantly more often than in normal animals), which some authors (Glagoleva and Chechulin, 1968) consider a sign of their division (Figure 2). A pronounced glial reaction of a productive nature is also characteristic of damage to nerve tissue.

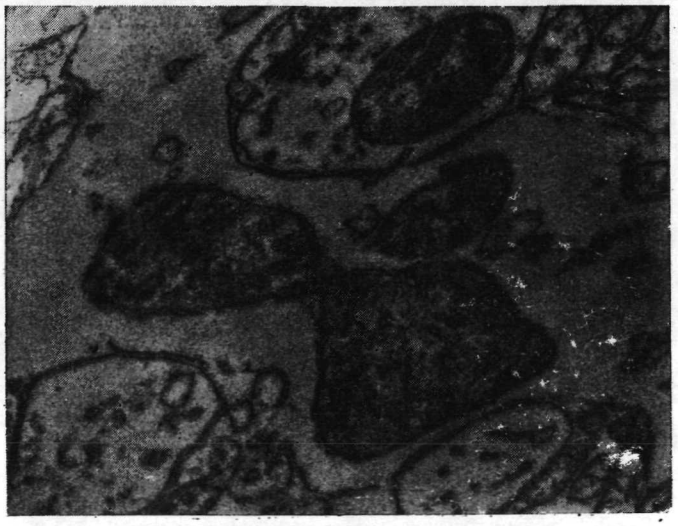

Figure 2. Electron Photomicrograph of the Hypothalamic Area of the Brain of a Rat. Pure oxygen at 6 ata. Intracellular edema and "tying off" of the mitochondria. 72,000 ×

During prolonged exposure to oxygen, peripheral nerves show swelling of nerve fibers, vacuolization and fragmentation of the myelin membrane, and a reduction of the activity of the oxidizing enzymes in the cells of the oligodendroglia are observed. /123

Structural changes which occur in animals under the influence of pure oxygen are also created in different parenchymatous organs (heart, liver, kidneys, and spleen).

In our experiments in a study of the effect of pure oxygen at 1 ata on white rats, we observed plethora of the superficial veins of

the heart after only three-six hours, directly beneath the epicardium, which was maintained for three days of the action when the animals began to perish. In addition, venous hyperemia set in later in deep layers of the myocardium. We did not notice significant changes in the metabolism of the muscle cells of the heart during the first three hours.

After six hours breathing pure oxygen the activity of the succinic acid dehydrogenase increased, while the activity of the lactic acid dehydrogenase decreased. Later on (after 24-72 hours) changes of a pathological nature gradually began to appear in the heart. Muscle cells in the outer layers of the myocardium and to a lesser degree in the musculus papillaris became adipose although dissimilarly in different animals. These processes were expressed more strongly in the right ventricle. The activity of this succinate dehydrogenase decreased, and we observed simultaneously an increase in the permeability of the mitochondrial membranes. The activity of the lactic acid dehydrogenase also revealed by histochemical methods, increased, although more significantly after 24 hours, and then decreased, remaining higher than the control level. Similar changes in the metabolism of the myocardium were detected by biochemical methods under conditions of subtoxic doses of oxygen (Vacca et al., 1966), and at increased barometric pressure (3 ata) in rabbits. The authors point out the abrupt increase in activity of lactic dehydrogenase and aldolase and the reduction in the amount of ATP. Thus, disturbances of cellular metabolism of the heart are related to a significant degree to damage to the mitochondria, to reduction of the intensity of processes of oxidative phosphorylation and compensatory activation of glycolysis.

Morphological changes in the heart during the use of oxygen under increased pressure are similar to the above-described changes and differ only quantitatively. We also noticed plethora of the vessels, small diapedesic hemorrhages in the myocardium and beneath the epicardium focal destruction of myofibrils, chromatolysis of the cells and sometimes vacuolization of the sarcoplasm of the muscle fibers.

It should be pointed out that the pictures of pronounced congested plethora appear in the most varied organs: in the liver, spleen, kidneys, intestine, etc. The liver generally reacts very quickly to a change in the composition of the gas medium. Thus, only three hours after breathing pure oxygen the animal /124 shows clear changes of metabolism in the liver. In comparison with the controls, the nuclei of liver cells showed a stronger reaction to protein as well as an increase in the dimensions of the nucleoli, which also confirm the intensification of the protein metabolism. Liver cells with forms of mitotic division are encountered approximately five to six times more frequently. Definite differences between the control and the test material are observed in the investigation of glycogen. In the controls, glycogen is found in the liver in larger amounts on the periphery of the lobules, and in the test glycogen is found in the middle and central sections of the lobules. In addition, we noticed a tendency towards reduction of activity of the succinate dehydrogenase in the central sections of the lobules.

Venous hyperemia arises very early in the system of hepatic veins (central and collective), which is reinforced later.

A reduction in the amount of RNA and protein in the liver cells of the peripheral sections of the lobules begins after six hours of the test, and an increase of glycogen in the middle and central sections. Mitotic division of liver cells is detected in the form of random finds. An increase in the activity of succinate dehydrogenase occurs approximately at this time.

After twelve hours signs of the turbid swelling type of protein dystrophy and hydrotypical changes of cytoplasm arise in the parenchymatous elements of the liver. Liver cells swell in all sections of the liver lobules, but especially strongly in the periportal zones, and the amounts of RNA and protein (and to a lesser degree, glycogen) decrease sharply.

At later periods (48 hours) the phemonena of protein dystrophy, vacuolization of cytoplasm and intracellular edema are reinforced when strong adiposity of liver cells occurs. Swelling and destructive mitochondria and myelin-like structures are often detected in electron photomicrographs and simultaneously the activity of enzymatic processes of biological oxidation

is decreased. Biochemical observations also confirm that hyperoxia leads to retardation of the enzymatic processes in the liver and reduction of oxygen absorption (Pfister et al., 1967). Activity of the glycolytic enzyme lactate dehydrogenase, on the other hand, increases. In this case we notice a difference in the activity of the enzyme in the center and on the periphery of the lobules more clearly than in the control, higher in the center and on the periphery of the lobules more clearly than in the control, higher in the center than on the periphery.

Death of the animals is often observed by the end of three days. The amount of glycogen in the liver is often reduced and processes which lead to adiposity of the periportal sections of the lobules are preserved.

Cases of the formation of small foci of necrosis and slight hemorrhaging /125
of a diapedesic nature are also encountered.

It is necessary to point out that the prolonged action of oxygen at reduced atmospheric pressure and for short times at high pressure (4-6 ata), as our experiments and also the literature data show (Schaffner, Felig, 1965), cause a similar change in the ultrastructures and metabolism of the liver. A decrease in the amount of glycogen, changes in the enzymatic activity of the cells increase in the dimensions of mitochondria due to their swelling, hypertrophy of the Golgi apparatus, formation of myelin-like structures, and appearance of numerous polysomes are usually observed to a greater or lesser degree. During the application of patently toxic exposures of increased oxygen pressure strong dystrophic changes of a hypoxic nature are created.

Without dwelling in detail on the histofunctional condition of other investigations in our experiments on tissues and organs (kidneys, spleen, skin, transversely striated muscle tissue), we nevertheless want to point out that the dynamics of changes detected in them basically corresponded to what was described above. Moreover, analysis of the observation in combination with the data in the literature permits us to isolate a number of general regularities, which are characteristic of hyperoxic damage to various organs.

In the first place, there is a pronounced vascular reaction to which we have referred more than once. It becomes evident that pure oxygen causes

damage to the endothelial cells (swelling, vacuolization, disturbance of the structure of mitochondria), expansion of the intracellular fissures leads to thickening or destruction of the basal membranes (Figure 3), and development of cellular, intracellular and perivascular edema. It is characteristic that these changes are detected in the most varied organs and tissues.

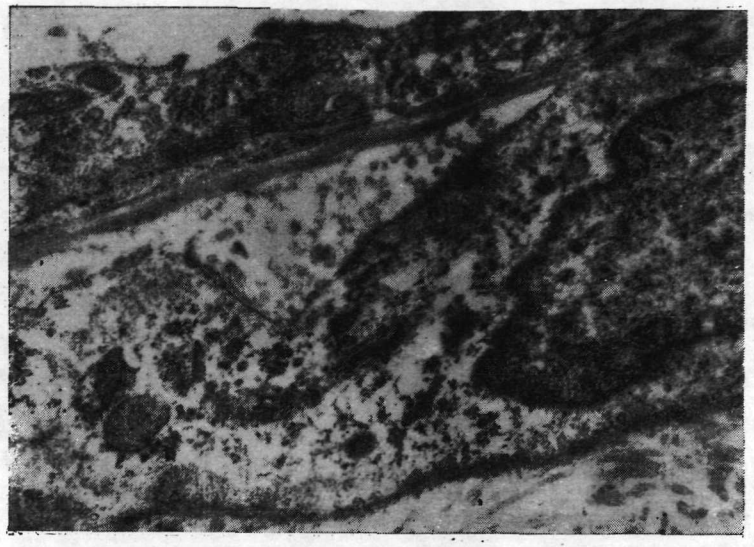

Figure 3. Electron Photomicrographs of a Section of a Capillary From Subcutaneous Connective Tissue of the White Rat. Pure oxygen at 1 ata. Exposure time two days. Destruction of the basal membrane and expansion of the intracellular fissures. 21,000 ×

In the second place, prolonged application of pure oxygen causes an increase of eosinophilic leucocytes in tissues; these leucocytes play an important role in protection of the organism during intoxication. Initially, the eosinophilic infiltrates are more often located perivascularly, which leads to the formation of massive accumulations of leucocytes around the vessels that surround them like "sleeves."

We noticed a similar reaction during the use of oxygen at increased pressure.

Thirdly, we noticed a definite tendency in the change of the ultrastructure and metabolism of cells of different organs. When maintaining animals in an atmosphere of pure oxygen at first (first six hours) activation of metabolism occurs without visible destruction of ultrastructures (activation of the oxidative enzymes increases, protein and nucleinic metabolism is reinforced,

and the amount of glycogen in a number of cells increases). A reduction of /126
intensity of the oxidative processes may initially begin in properly oxygenating cells (in the lungs, cerebral cortex, middle sections of the liver lobules), but later the activity of the enzymes of the Krebs cycle increases as a rule. At later periods (after twelve hours of exposure) we noticed phenomena of a pathological nature in the pattern of adiposity of cells, a reduction of the amount of RNA and protein in them, disturbance of the ultrastructure of mitochondria (swelling, reduction of the number of cristae, destruction of the mitochondrial membranes, formation of myelin-like forms). Simultaneously the activity of the mitochondrial enzymes decreased and the activity of the glycolytic enzymes increased. A similar regularity was described above in different organs and quite clearly is continued in the transversely-striated muscle tissue (Figures 4 and 5). Because there is a great amount of data in the literature on the formation of lipid peroxides in an organism during hyperoxia (Webb et al., 1966; Wollman, 1966 and others) and in view of the fact that the mitochondrial enzymes are suppressed according to the results of cytochemical /127
investigations, we can assume a primary affection of the mitochondria as a result of oxidation of the lipids which form them.

Figure 4. Electron Photomicrograph of Transversely Striated Muscle Fiber from Anterior Peritoneal Wall of White Rat. Pure O_2 at 1 ata. Exposure, two days. Destruction of cristae of mitochondria and accumulation of electronically dense granules. 72,000 ×

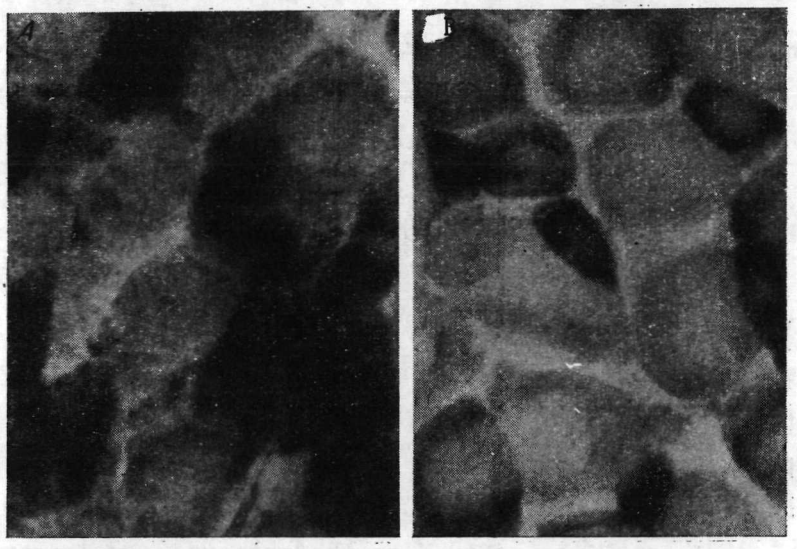

Figure 5. Reduction in Activity of Succinate Dehydrogenase in the Transversely-Striated Muscle Tissue of the Anterior Peritoneal Wall of a White Rat. Pure oxygen at 1 ata. Exposure time, one day. Objective 9×, ocular 12.5 ×. A, Control; B, Experimental.

Thus, disturbances of a vessel wall, which makes diffusion of oxygen in a tissue difficult, and the immediate toxic injury of cells creates conditions for the development of a hypoxic state. Therefore the simultaneously observed strengthening of the activity of the glycolytic processes can be seen as a compensatory reaction of cells in response to hypoxia. However, we also noticed a suppression of the activity of the glycolytic enzyme, lactic acid dehydrogenase, in a number of cases during the investigation of animals kept 2.5 to 3 days in an oxygen atmosphere. The mechanism of this phenomenon has not yet been clarified. It is supposed that peroxides which are formed during the action of oxygen cause an immediate inactivation of a number of enzymes (Gershenovich, Bronovitskaya, 1955; Frejaville, 1967).

Certain specific characteristics of the change of both the structure and metabolism of various tissues and organs become increasingly evident in addition to the above-described general typical cellular reactions to hyperoxia. An important peculiarity consists in the fact that a qualitatively monotypical reaction quantitatively appears nonuniformly. Thus, for example, the greatest

morphological changes during breathing of pure oxygen at normal barometric pressure are observed in the lungs, significant changes are observed in the liver and less pronounced ones in other organs. A similar regularity was also noticed in a tissue culture (Heppleston and Simnett, 1964). During cultivation of a tissue culture of the lungs, kidneys, prostate and epididymis in a gas mixture of 95° O_2 and 5% CO_2 at a pressure of 1 to 6 ata for three days degenerative changes arise in all of the organs (karyolysis, suppression of mitoses, etc); however, the most pronounced changes occur in the alveolar tissue of the lungs. /128

The varying sensitivity of oxygen is also noticed in comparing histological tissues. This yields the following picture. The parenchymatous epithelium is affected more than the multilayer flat keratosic epithelium of the skin, and the cardiac muscle is more sensitive than the smooth muscle tissues of the vessels and bronchi. The adipose cells, fibroblasts and endothelium of the vessels in the connective tissue react more strongly. Evidently the lymphoid tissue is suppressed to the greatest degree among the formed elements of the blood.

In conclusion we would like to note that in spite of the long history of the problem of hyperoxia, the morphology and cytochemistry of oxygen damage have still been inadequately investigated. Such organs as the liver, lungs, heart and kidneys have been more or less well studied. There are practically no works on the histophysiology of the endocrine system during hyperoxia. We must carefully explain the structure and metabolism of different sections of the brain, of course in comparison with the corresponding physiological indices. This area has practically not been studied at all, but might yield a great deal of valuable information. It would be extremely interesting to trace in detail the condition of the lymphoid tissue under conditions of hyperoxia, because the latter is very sensitive to the action of oxygen (Portugalov et al., 1968). And, finally, much is still unclear in the mechanisms of the toxic effect of oxygen. All these problems demand further serious analysis. /129

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ACTION OF HYPEROXIA ON CONNECTIVE TISSUE

L. L. Shimkevich

ABSTRACT. A general morphological, electron microscopic and cytochemical study of subcutaneous connective tissue of white rats while keeping them under conditions of increased partial pressure of oxygen was conducted. A short-term (six hours) action of pure oxygen at 1 ata caused activation of metabolism of the connective tissue cells (buildup of glycogen, increase of activity of the oxidative enzymes) without disturbance of the ultrastructures. Noticeable pathological changes arose after twelve hours, later they advanced and appeared as destruction of cellular organelles, as suppression of cellular metabolism, and partially as a reduction in the activity of the oxidative enzymes. Destructive changes of collagenic fibers were noticed. Data are also present on the structure and cytochemistry of connective tissue under other conditions of hyperoxia and 70, 50, 100% at an "elevation" of 2,400 m). The investigation has importance for development of an optimum atmosphere for cabins of space vehicles and for medical practice.

The reaction of connective tissue to hyperoxia has been studied very little. /130 There exist only isolated reports on the effect of increased partial pressure of oxygen on the condition of collagen. It has been shown that oxygen suppresses the formation of collagenic fibers and osteogenesis in a tissue culture (Shaw, Bassett, 1967); initially both the synthesis and the decomposition of collagen increase with an increase in the concentration of oxygen, but later the decomposition of collagen prevails over its synthesis (Stern et al., 1966). While keeping experimental animals in an artificial gas medium with an oxygen concentration above 35% we noticed substantial changes in the chemical composition of collagen, which formed in a skin wound and in a granuloma (Kulonen et al., 1967).

The broad distribution in the organism, its important role in maintaining constancy of internal environment and the participation of connective tissue in many pathological processes convinced us to examine cellular elements and noncellular structures of connective tissue in tests carried out together with F. V. Babchinskiy, Ye. F. Kotovskiy and others in order to study the effect of

different conditions of hyperoxia on the duration of life, on physiological functions and on the condition of organs and tissues of animals. We conducted the experiments on white male rats. The animals were kept in a special chamber provided with systems of ventilation, gas purification, water supply and devices for controlling the physical parameters of the gas medium.

In all, four series of tests were conducted. In the first series the animals were subjected to the action of an atmosphere of 100% oxygen at normal barometric pressure (1 ata); in the second series of tests there was 70-80% oxygen in the mixture with nitrogen at 1 ata; in the third series there was 50% oxygen in the mixture with nitrogen at 1 ata; in the fourth series there was 100% oxygen with rarefaction of 567 mℓ Hg, (altitude 2,400 m). The object of the investigation was subcutaneous porous fibrous connective tissue, and also a connective tissue section (the papillary and reticular layers) of the skin of the anterior abdominal wall of the rats. In the first series the animals were killed after 3, 6, 12, 24, 48, 60 and 72 hours, and in the remaining series on the 1, 3, 5, 10, 15 and 20th days from the beginning of the experiment. The series paraffin microscopic sections were treated by common morphological and histochemical methods (hematoxylin-eosin, azur II-eosin, Schick reaction, toluidine blue at different pH values), and in sections prepared in a cryostat, the activity of the oxidative enzymes appeared (succinic and lactic acid /13
dehydrogenase). In the first series of tests an electron microscopic investigation of connective tissue was conducted. In this case the (first series of material was fixed in osmium tetroxide by the Caulfield method and was cast in methacrylates. The microscopic sections prepared on the LKV-4800 ultramicrotome were contrasted by the Reynolds method and studied in the JEM-6 AS electron microscope.

When the animals were kept in an atmosphere of pure oxygen (first series of tests) for three hours the general morphology of the connective tissue did not change. The difference from the control (animals under normal conditions) is the increase in buildups of eosinphilic leucocytes, which were located perivascularly.

A slight increase was also noticed in activity of succinic acid dehydrogenase in the cellular elements of the connective tissue. After six hours, clear granules of glycogen appeared in the fibroblasts and macrophages; this is almost never seen in rats under normal conditions. The activity of both succinic dehydrogenase and lactic acid dehydrogenase was increased in the majority of cells. Twelve hours after the beginning of the action of the oxygen, expansion of the vessels occurred, and we sometimes observed a weak edema and eosinophilic infiltrates. Fine granules of glycogen appeared in the adipose cells located around the vessels, and the amount of highly sulfated mucopolysaccharides decreased; their degranulation is intensified. A high activity of the oxidative enzymes appears in all the cells of the connective tissue, and during electron microscopic investigation there is a slight swelling of the mitochrondria and occasional degenerative changes in them.

Pronounced morphological changes occur in a number of structures by the end of the first day. This happens in the vessels in particular. The endothelial cells swell and contain a large number of vacuoles, while the membranes are broken down and intensively Schick-positive. The Schick-positive reaction of collagen fibers is also strengthened, which indicates their disorganization. The content of glycogen increases in the cytoplasm of the connective tissue cells and also in the leucocytes which are in both the lumina of the vessels and in the tissue. The activity of the succinate dehydrogenase sharply decreases, and in certain cells it is not at all detectable. At the same time the activity of the lactic acid dehydrogenase does not change substantially.

After two days the blood vessels are greatly expanded and overfilled with blood, pronounced vacuolization takes place in the endothelium, and perivascular edema is manifested to a high degree. Electron microscopic investigation shows a sharp increase in the amount of pinocytotic vesicula in the endothelial cells, destruction of the mitochondria in them and formation of /132
myelinic shapes. The basal membranes are thickened and undergo disintegration in different places (Figure 1). The intercellular spaces are frequently expanded. The number of eosinophilic leucocytes is increased, but they are

diffusely positioned in the tissue, and not perivascularly as during the previous time periods. Pronounced degranulation of the adipose cells is noticed. The amount of glycogen in comparison with preceding time periods decreases in the cellular elements of the connective tissue, and the activity of all the enzymes is reduced (Figure 2). Electron microscopy of the fibroblasts reveals the greatest changes in the mitochondria, in which swelling, disorientation of the inner membranes, clearing of the matrix, and sometimes destruction of the organelles are observed (Figure 3). However, in addition to the above-described changes, mitochondria which contain a great number of densely packed cristae are encountered. This is in conformity with histochemical data which confirm the presence in the fibroblasts of single but nevertheless clear granules of the enzymatic activity of the mitochondrial enzyme succinate dehydrogenase. The number of such granules, although less than in the control, is somewhat higher in comparison to the preceding time period. It is completely possible that the above-described observations are a reflection of the partial adaptation of cells to unfavorable conditions of existence. /133

Figure 1. Electron Photomicrograph of a Section of a Capillary From Subcutaneous Connective Tissue. 100% O_2 at 1 ata. Exposure time of 48 hours. Intensification of pinocytosis in an endothelial cell, damage to the structure of mitochondria and thickening of basal membrane. 37,000 ×

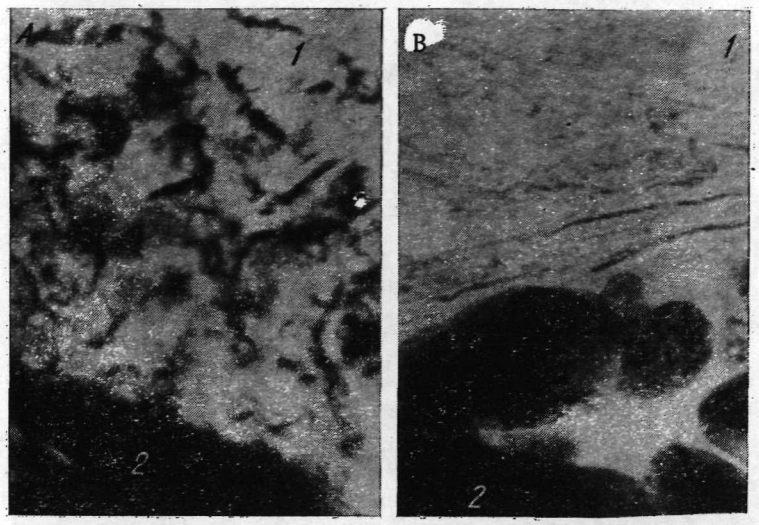

Figure 2. Photomicrograph. Reduction of activity of lactic acid dehydrogenase after 48 hours of the action of pure oxygen in disintegrated connective tissue (1) during the absence of pronounced changes in transversely striated muscle tissue (2). A, Control; B, Experimental. Objective 20 ×, ocular 12.5 ×

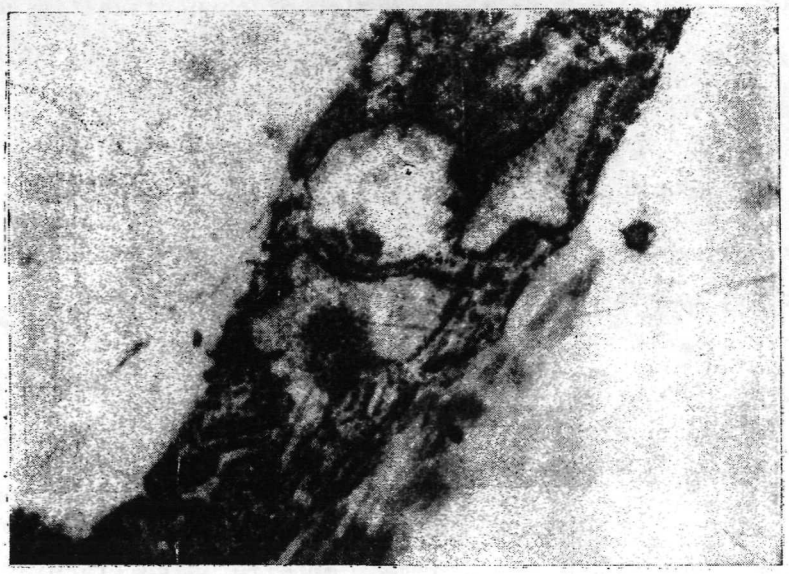

Figure 3. Electron Photomicrograph of a Section of Fibroblast from Subcutaneous Connective Tissue. 100% oxygen at 1 ata. Exposure time 48 hours. Explanation in the text. 23,000 ×

This time period (48-60 hours) is evidently critical, when tissue metabolism is abruptly distorted and irreversible structural changes occur. Destruction of the mitochondria, reduction of glycogen and a number of other disorders of cell metabolism reveal the onset of tissue hypoxia (Glagoleva,

Chechulin, 1968), which increases in later periods. A characteristic feature of this period is also the increased storage of fat in the cells and the intensive formation of fatty tissue. The latter is supported not only by the morphological observations but also by the abrupt increase in activity of the oxidative enzymes.

After three days the morphological changes in the connective tissue basically correspond to what has been described above, and in a number of cases an increase in the activity of lactate dehydrogenase is even noticed. Adventitial cells, fibroblasts, macrophages and especially leucocytes often contain glycogen granules. The collagen fibers appear swollen and intensively Schick-positive.

On the whole one can note that the reaction of connective tissue of /134
the derma begins earlier than the porous connective tissue, and the harmful action of oxygen shows up in it to a lesser degree. Evidently this is related to peculiarities of the topography of these tissues in the organism and to different conditions of oxygenation.

When animals are kept in an atmosphere of 70% oxygen in a mixture with nitrogen (II series), for the first three days no visible morphological changes were detected, with the exception of a slight perivascular infiltration of eosinphilic leucocytes. Slight edema of intercellular substance appeared after five days, in addition to swelling of collagen fibers and a reduction in the amount of heparin in the adipose cells. The Schick reaction of the cytoplasm was intensified in the fibroblasts and macrophages and the amounts of nucleic acids and amino acids increased. Enlargement of diformazan granules occurs when setting up a succinate dehydrogenase reaction.

On the fifteenth day significant structural changes developed in the connective tissue. The number of eosinphils around the vessels increases, and there is swelling of the endothelial cells and their desquamation in the lumen of vessels. The dimensions of the cytoplasm in the fibroblasts and microphages increased; and at times we observed vacuolization, and glycogen granules /135
appeared. The enlarging of the diformazan granules which occurs during the appearance of succinate dehydrogenase indicates not only an increase in the enzymatic activity but also an increase in the permeability of the mitochondrial

membranes. Activity of lactic acid dehydrogenase did not change substantially. The adipose cells contain a smaller number of granules with highly sulfated mucopolysaccharides of heparin type, and vacuoles are sometimes encountered in their cytoplasm. We observed swelling of the collagen fibers and intensification of their Schick-positive reaction in the reticular layer of the skin.

After twenty days destructive changes in the endothelium and basal membranes of the vessels. The endothelial cells in the arteries become long, desquamate, and often completely separated from the basal membrane at a great distance. The adipose cells vigorously degranulate, and the macrophagic reaction is intensified. The activity of the succinate dehydrogenase in all the cell elements of the connective tissue is reduced significantly, while the activity of the lactate dehydrogenase changes very little.

In the III series of tests (50% hyperoxia) noticeable differences from the control set in on the eleventh day from the beginning of the experiment. The cell reaction is characterized by an increase in the amount of glycogen and by a certain swelling of the cytoplasm. In the adipose cells the number of granules with highly sulfated mucopolysaccharides is decreased. By the twentieth day a more pronounced affection of the vessels and the connective tissue cells is noticed. In its morphological details this stage reminds one of the fifteen-day exposure with 70% oxygen. We also noticed an increase in the activity of succinate dehydrogenase, which was especially significant in the macrophages and lymphocytes which infiltrate the tissue. An increase in the activity of the oxidative enzymes in the fatty tissue and an increase in the number of fatty cells are characteristic for the given time period.

Keeping the animals in an atmosphere of pure oxygen at reduced atmospheric pressure (IV series of tests) for a prolonged time did not cause significant disorders of the structure and metabolism of the connective tissue. Even after fifteen days of exposure observed were only a small swelling of the endothelium, an increase of the eosinophilic infiltration and some activization of the connective tissue cells (increase in the dimensions of the cytoplasm, accumulation of glycogen), although the activity of the oxidative enzymes did not substantially change. Nevertheless, a pronounced disturbance was noted of the

morphology of cells and a reduction in the activity of the dehydrogenases during a study of the other tissues of the skin of these animals.

The cited observations confirm the substantial effect of a hyperoxic medium on the structure and metabolism of connective tissue. In this connection the concentration of oxygen in the surrounding atmosphere and the length of the /136
exposure time are of significant importance.

The short-term (six hours) action of pure oxygen at normal barometric pressure induces activation of metabolism of the connective tissue cells (accumulation of glycogen, increase in activity of oxidative enzymes) without disturbing the ultrastructures. Noticeable pathological changes are observed only after twelve hours and then progress. At this time there is a destruction of the cellular organelles and particularly a destruction of the mitochondria, especially pronounced 48-60 hours after the beginning of the experiment. Affection of the mitochondria leads to destruction of the normal course of the biochemical reactions of tissue respiration, which is accompanied partially by suppression of the activity of the succinate dehydrogenase. The enzymes of glycolysis are evidently more resistant to the toxic action of the oxygen because the activity of the lactic acid dehydrogenase is decreased somewhat later.

Significant vascular changes are characteristic for the hyperoxic affection of the connective tissue: damage to the endothelial cells and basal membranes, perivascular edema, and also eosinophilic and lymphoid infiltration of the intercellular substances. Simultaneously observed is an increased degranulation of the adipose cells with excretion of heparin-type highly sulfated mucopolysaccharides. Some authors (McGovern, 1955; Riley, 1963) think that during the action of harmful agents on connective tissue degranulation of the adipose cells occurs, their substance is secreted to the surface of the endothelium and the permeability of the wall of the vessel changes, which leads to exudation of the fluid into the tissue. However, there is another opinion (Drennan, 1951; Wegelius, Asboe-Hansen, 1956), that the permeability itself of the capillaries is a stimulus for liberation of heparin by the adipose cells, and degranulation is a reaction to the increase of water in the tissue; mucopolysaccharide, which

is discharged from the adipose cell, binds the tissue water. Dynamics of the changes which we found in our experiments support the latter supposition. In such an instance the reaction of the adipose cells should be viewed as protective and intended for regeneration of the disturbed metabolism of the tissues. It is true that during prolonged exposure to pure oxygen, its immediate toxic action shows up clearly in the adipose cells (vacuolization of the cytoplasm, suppression of the oxidative enzymes). As a result a unique "vicious circle" and correspondingly, an increase of pathological reaction is created.

The destructive changes of the collagen fibers which we observed in our experiments (most pronounced at 100 and 70% hyperoxia) are also confirmed by literature data (Shaw et al., 1967; Stern et al., 1966), although the mechanism for these changes has not yet been explained. It is supposed (Kulonen et al., 1967), that oxygen in an increased concentration may affect the amount of oxyproline in the collagen which leads to a reduction in the resistance of the fiber to rupture.

Hypoxia, as is known, is a factor which in many cases stimulates the development of connective tissue, especially when atrophy of other tissues sets in. The activation of connective tissue cells which arises during a small degree of hyperoxia is related chiefly to carbohydrate and energy metabolism. /137 It is highly possible that the increased amount of oxygen in the surrounding atmosphere may suppress processes of scarring and sclerosis. It would be interesting to check this supposition on corresponding pathological models.

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GAS EXCHANGE AND ELECTRICAL ACTIVITY OF THE SKELETAL MUSCULATURE OF ANIMALS IN A HELIUM AND OXYGEN MEDIUM

G. V. Troshikhin

ABSTRACT. The gas exchange, electrical activity of the skeletal musculature (EAM) -- the heat regulating tonus, and the rectal temperature for rats which were kept for one hour in the air, in a helium-oxygen mixture and again in air, were studied.

For the animals which were kept in the helium-oxygen atmosphere at room temperature (20-22°) a definite increase is noticed in the level of gas exchange, an increase of the EAM and a drop in rectal temperature. Transition to air respiration brought about normalization of almost all the functions examined, but for an hour the heat balance, evidently, was not completely restored because the body temperature of the rats did not reach the starting level and on the basis of EAM indices remained at an increased level.

Increasing the temperature of the helium-oxygen mixture by 5° (25-27°) in comparison with that of air (20-22°) led to a small decrease of the EAM indices, the gas exchange and body temperature. Replacing the "helium" respiratory mixture by air and the hour-long confinement in an ordinary atmosphere at the starting temperature almost completely normalized the heat-regulating reactions.

The data obtained permit the idea that a more accurate determination of a comfortable temperature range in the helium-oxygen atmosphere requires that the heat regulating activity of the skeletal musculature also be considered in addition to measuring rectal temperature.

The use of helium-oxygen mixtures for respiration in clinical conditions /137
and in diving practice (Barach, 1934; Raymond et al., 1968) has increased the interest in studying the effect of helium on the physiological functions of an organism. The feasibility of prolonged confinement of man and animals in a helium-oxygen atmosphere has been established and convincing arguments on the efficacy of using helium as one of the components of a gas medium in the cabins of space vehicles have been introduced (Boriskin et al., 1963; Dianov, Kuznetsov, /138
1963; Savin, 1965; Bond, 1963; Hamilton et al., 1966; Robertson et al., 1966). It is known that because of the high heat conductivity of helium an atmosphere

containing it has a cooling action on man (Bonura et al., 1967; Epperson et al., 1966; Fox et al., 1966). However, peculiarities of the heat regulating function of an organism under these conditions have been insufficiently studied. The present article will examine this problem.

Experiments were conducted on forty Wistar-strain male rats which weighed 220-320 g. The gas exchange, electrical activity of the skeletal musculature (EAM) -- the heat regulation, tonus, and rectal temperature of animals in the air and in a helium-oxygen mixture (21% O_2 and 79° He) were studied. The temperature of the medium was maintained at a given level by the aid of thermostats.

The oxygen consumption and discharge of carbon dioxide was determined in a 20 l capacity pneumatic chamber. The muscular activity was recorded electrophysiologically. Mishel' clamps were used as electrodes and biocurrents were recorded from the neck muscles which are the most active in terms of heat regulation (Ivanov, 1965). The EAM changes were recorded automatically by a special unit which consists of a UDT-1-01 amplifier, a PS-100 pulse counter and a V. A. Kozhevnikov system integrator (1963). The number of EAM peaks and their area were computed for one minute in arbitrary units. The body temperature was measured rectally by an electric thermometer.

The order of tests was as follows. A rat with electrodes attached and a temperature sensor inserted into the rectum was placed into a pneumatic chamber, in which the animal was in an atmosphere or air, helium-oxygen mixture and again in air for successive one-hour periods. Air samples were taken from the chamber at the beginning and at the end of each one-hour period and were analyzed in a Haldane apparatus. Calculation of the gas exchange was carried out in the normal manner for such conditions. (Ol'nyanskaya, Isaakyan, 1959). During the exposure period, the number and area of EAM peaks and rectal temperature every five minutes were recorded. The data obtained were analyzed statistically.

In tests of series I the temperature of the air and the helium-oxygen mixture was maintained at a constant level (20-22°). The investigation showed that the gas exchange (Figure 1) and the EAM (Figure 2) of the rats increased

in the helium-oxygen atmosphere. Oxygen consumption increased on the average by 27.6% (r = 0.001), and output of carbon dioxide increased by 21.2% (r = 0.05) in comparison with the starting values. Muscular activity, according to the number of electrical peaks, on the average increased by 25.7% (r = 0.005), and measured according to their area it increased by 55.6% (r = 0.001). Concurrently rectal temperature decreased (Figure 3); on the average the drop was 9.1% (r = 0.001) of the original value.

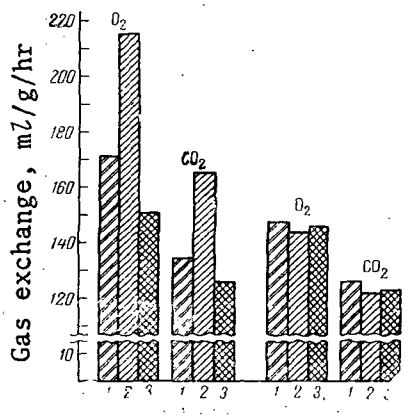

Figure 1. Gas Exchange Changes During Exposure of Animals in Air and Helium--Oxygen Media. A, Temperature of the air and the helium-oxygen medium 20-22°; B, Air temperature 20-22°; temperature of helium-oxygen medium 25-27°; 1, Starting value in air; 2, The same in helium-oxygen medium; 3, the same in air.

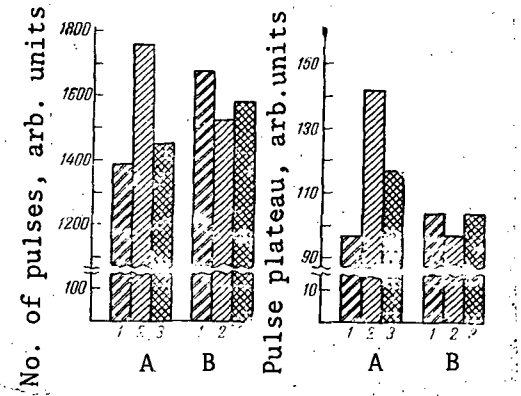

Figure 2. Changes in Electrical Activity of the Musculature During Exposure of Animals in Air and Helium-Oxygen Media. Callouts are the same as in Figure 1.

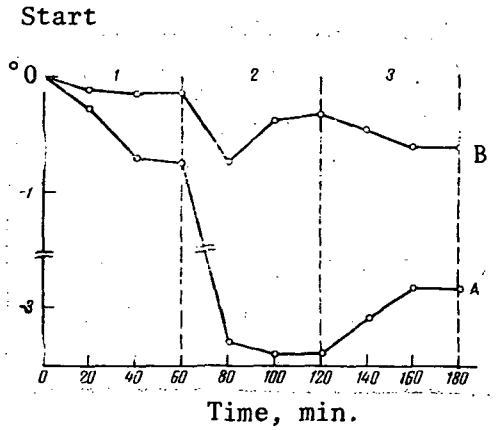

Figure 3. Relative Changes in Rectal Temperature During Exposure of Animals to Air and Helium-Oxygen Media. Callouts are the same as in Figure 1.

After replacing the "helium" atmosphere by air the gas exchange and EAM based on the number of electrical peaks decreased approximately to the level of the original values, except /139

for the area of the pulses. The level of the latter in the air medium exceeded the initial control figures by 18.2% ($r = 0.01$). The body temperature of the rats in the reaction period following the one-hour confinement in air increased slowly, in all by 1% (the difference remains statistically verifiable).

Thus, a definite increase was noticed in the level of gas exchange, an increase of the EAM and a drop of rectal temperature for rats in the helium-oxygen atmosphere at room temperature. /140

Transition to air respiration brought about normalization of almost all the functions studied, but for one hour the heat balance evidently did not regenerate completely, because the body temperature of the animals did not reach the initial level, and one of the EAM indices remained at an increased level. Obviously, the principal reason for the observed shifts is cooling of an organism in a "helium" medium.

According to data in the literature, the cooling action of a helium-oxygen atmosphere disappears when the temperature of the medium is increased by 3-5° in comparison with the air temperature (Konza, 1965; Leon, Cook, 1960; Rhoades et al., 1967). The authors' were interested in finding out whether such a difference in temperature was sufficient for balancing the heat-regulating reactions of animals. With this purpose in mind a series of tests II were set up in which the temperature of the "helium" medium exceeded the temperature of the air medium by 5°. The air temperature was maintained at a level of 20-22°, and that of the helium-oxygen mixture was respectively at 25-27°.

It turned out that under the given conditions the general gas exchange of the rats in the helium-oxygen atmosphere increased slightly. Oxygen consumption decreased by 2.7% ($r > 0.05$), and discharge of carbon dioxide decreased by 3.1% ($r = 0.05$). The EAM indices also decreased. The number of EAM pulses turned out to be 9.4% less in comparison with the starting values ($r = 0.001$), and the area was 5.9% less ($r = 0.01$). Rectal temperature for the rats in the helium-oxygen atmosphere fell by 0.4° ($r = 0.01$).

Transition of the animals to respiration by air (at a temperature of 20-22°) caused an increase in the level of gas exchange and EAM. It is true that when the area of the EAM peaks increased to the initial level the number of /141
pulses was 5.9% reduced ($r = 0.005$).

The total gas exchange of the animals also remained somewhat reduced. The oxygen consumption was 1.4% less than the original level ($r > 0.05$), and the discharge of carbon dioxide was 3.1% less ($r > 0.05$). Rectal temperature for the one hour confinement in air did not change on the average.

The investigation showed that increasing the temperature of the helium oxygen mixture by 5° in comparison with the air (in the range 20-22°) brought about a small reduction in the EAM indices, gas exchange and body temperature. Replacing the "helium" respiratory mixture by air and the one-hour long confinement in a normal atmosphere at a starting temperature did not completely normalize the heat regulatory reactions.

A number of investigators have noticed a 15-23% increase of gas exchange in rats in a helium-oxygen atmosphere at room temperature (Leon, Cook, 1960; Rhoades et al., 1967). We observed a similar effect in our tests. The level of tissue metabolism in vitro, judging by certain data, does not change (Lang, (Wright et al., 1964). It is thought that the increase of gas exchange in the helium-oxygen atmosphere at normal pressure occurs as the result of cooling of the organism due to the high heat conductivity of helium (Dianov, 1967; Troshikhin, 1966; Leon, Cook, 1960; Rhoades et al., 1967). It is known that in order to maintain temperature homeostasis in animals during cooling the level of chemical heat regulation by means of an increase of the EAM increases, and is accompanied by an increase in the gas exchange level (Ivanov, 1965). Similar relationships appeared in our experiments, when the level of the heat regulating tonus and gas exchange increased at an identical temperature of air and helium-oxygen atmosphere.

Increasing the temperature of the helium-oxygen medium in the II series by 5° in comparison with the air, obviously, reduced the heat exchange due to physical factors (reduction of the temperature gradient of skin and medium), which was accompanied by a small drop in the gas exchange level and the EAM.

Bearing in mind that an air temperature of 20-22° is not thermo-neutral for rats (Ivanov, 1965; Slonim, 1952), we can assume that the heat-regulating reactions in animals under the given conditions are under definite stress. Replacing the air by the helium-oxygen mixture at a temperature of 25-27° removes this condition. It is likely that at such a temperature of the "helium" medium it is "warmer" for the rats than at a corresponding temperature (20-22°) in air, because the heat-regulating tonus of the muscles and their gas exchange decreased. The slight reduction of rectal temperature in these tests could have been caused by a certain inertia of temperature shifts in comparison with the EAM and the gas exchange. The data obtained lead us to believe that a more accurate determination of a more comfortable temperature range of a helium-oxygen atmosphere for man would require consideration of the heat-regulating activity of the skeletal musculature in addition to measurements of skin and rectal temperatures. /142

Comparison of the EAM indices in the helium-oxygen atmosphere during temperature fluctuations in the range 25-27° provides comparatively larger changes of the heat-regulating activity of the muscles during a 1° drop. It can be assumed that the thermoneutral range is narrower in comparison with the air for rats in a "helium" medium. We do not rule out the fact that the cause of this phenomenon is related to the smaller heat capacity of helium relative to nitrogen. The complex regularities of heat exchanges in organisms in a helium-oxygen atmosphere require a further comprehensive investigation.

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STUDY OF THE PHYSIOLOGICAL EFFECT OF REPLACING ATMOSPHERIC NITROGEN IN THE AIR WITH INERT GASES UNDER CONDITIONS OF OXYGEN INSUFFICIENCY AND INCREASED CONCENTRATIONS OF CARBON DIOXIDE

M. M. Osipova and A. G. Dianov

ABSTRACT: In experiments with rats it is shown that replacing atmospheric nitrogen with argon in hermetically enclosed chambers at -22° did not affect the basic physiological function and longevity of the animals (in its thermal conductivity argon does not substantially differ from nitrogen). This confirmed the supposition that an increase of longevity of animals in hermetically enclosed chambers with a helium-oxygen atmosphere is determined by the high thermal conductivity of helium in comparison with nitrogen.

In the preceding investigations (Dianov, 1967) the physiological effect of /143
replacing atmospheric nitrogen by helium with the simultaneous action of oxygen deficiency on the organism and increased concentrations of carbon dioxide was studied. Experiments were conducted on animals in a helium-oxygen medium under conditions of a hermetically sealed chamber without regeneration of the atmosphere, when as a result of the vital activity of the organism there occurred a gradual reduction in the concentration of O_2 and an increase in the concentration of CO_2. It was established that replacing the atmospheric nitrogen by helium under the stated conditions at a temperature of 22° led to statistically reliable increase in the longevity of the animals by 42%. Simultaneously it was noticed that natural hypothermy, which developed due to the effect of hypoxia and hypercapnia, was a great deal more pronounced in the helium-oxygen medium than in the air. The reduction of body temperature in an ordinary air medium, as is known, led to reduction of oxygen consumption and increase of longevity of the animals in hermetically sealed chambers without regeneration of the atmosphere (Konstantinov, 1955). In this connection it was hypothesized that a substantial increase in the longevity of the animals in the helium-oxygen medium was related to the increased heat transfer of the organism which in turn was conditioned by the high thermal conductivity of helium in comparison

with that of nitrogen. Analysis of the experimental data obtained with higher ambient temperatures (27-40°) also led to this conclusion.

In order to check our hypothesis under similar conditions a series of experiments was conducted in which atmospheric nitrogen was replaced by argon /144
in the atmosphere of a hermetically-sealed chamber. Selection of this inert gas was determined by the fact that it does not substantially differ from nitrogen in its thermal conductivity.

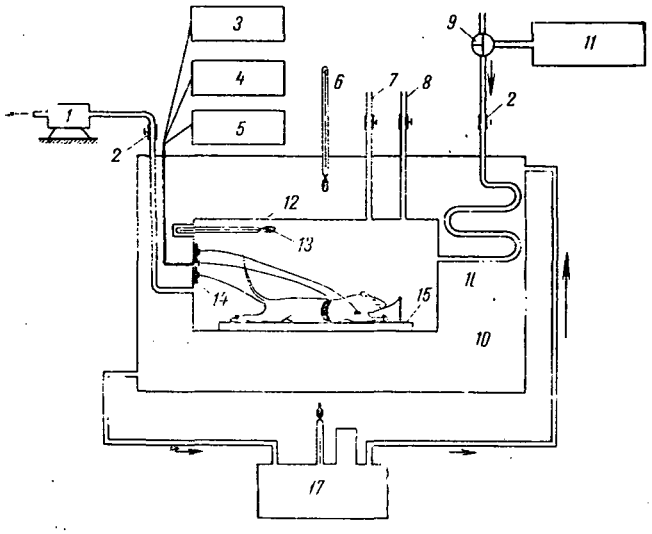

Figure 1. Basic Layout of the Experimental Unit. 1, Air pump; 2, Clamps; 3, Electrocardiograph; 4, Device for recording respiratory movement; 5, Device for measuring body temperature; 6, Thermometer; 7, Connecting pipe for taking gas samples; 8, Connecting pipe for measuring pressure in the chamber; 9, T-valve; 10, Thermostatic chamber; 11, Rubber bag with argon-oxygen mixture; 12, Hermetic chamber; 13, Thermometer in the chamber; 14, Intermediate socket; 15, Support for restraining the animal; 16, Coiled pipe; 17, Thermostat.

Experiments were conducted on white male rats weighing 115-120 g by using a device whose basic layout is shown in Figure 1.

Before the beginning of each experiment the restrained animal was placed with sensors attached in a hermetic chamber with volume of 1,190 cm^3. This chamber was made of Lucite, which made it possible to observe the animal in the course of the experiment. After hermetic sealing, the chamber with the animal inside was placed in a thermostatic chamber, which was also made of lucite and designed to maintain a constant temperature of the gas medium in the hermetic chamber. This was achieved by the fact that water with its temperature maintained at a given level circulated continually around the hermetic chamber.

of its temperature were ensured by the aid of a TS-24 thermostat. Ventilation /145
of the hermetic chamber by air or the argon-oxygen mixture was achieved by the aid of an air pump which had an output of 3 l/min. Before entering the hermetic chamber, the ventilating gas mixture passed through a copper coil where it acquired the given temperature.

While conducting the control experiments in a normal air medium, the hermetic air chamber was ventilated with air for 30 minutes while the experimental animal was inside, in order that the assigned temperature conditions become stable inside the chamber. While conducting the experiments in which nitrogen was replaced by argon, the chamber was ventilated with air for the first fifteen minutes, and then for the following fifteen minutes it was ventilated with the argon-oxygen mixture which consisted of 20.5-21% oxygen and 79-79.5% argon. The transition from ventilation of the chamber with air to ventilation with the argon-oxygen mixture was achieved by aid of the T-valve. As the time passed the gas medium in the hermetic chamber hardly differed in its composition from the argon-oxygen mixture which was used for ventilation. In all of the experiments the pump was disconnected after a thirty-minute period of ventilation of the chamber, and the chamber was hermetically sealed by closing the clamps on the inlet and exhaust rubber tubes.

In the course of the experiment the rate of respiration, body temperature, and the rate of heart contractions for the animals was studied and we also determined the survival time from the moment of hermetic sealing of the chamber until cessation of respiration. The rate of heart contraction was determined by recording the biocurrents of the heart muscle on a two-channel electrocardiogram. On this device the respiratory movements of the animal by the use of a special attachement were recorded. Rectal measurements of body temperature of the rats were taken by the aid of a microthermistor, which was connected to a bridge network.

The phsiological indices were registered every five minutes during the experiment. The last recording of the rate of heart contractions and measurement of body temperature were taken at the moment of cessation of respiration.

The experiments were conducted with gas medium temperatures in the chamber of 22, 27, 31, 36 and 40°. Ten experiments were conducted for each of indicated temperature values (fifty experiments in all).

Data[5] on the longevity of the animals in the hermetically-sealed chamber under conditions of the argon-oxygen medium are presented in Table 1.

TABLE 1. DURATION (IN MIN) OF RATS IN HERMETICALLY SEALED CHAMBER* /146

Composition of gas medium	Temperature of the gas medium (°C)				
	22	27	31	36	40
Air	42,2±1,2	37,0±1,1	32,4±0,9	27,3±0,7	16,0±0,5
Helium-oxygen medium	59,9±3,5 P<0,001	48,2±2,7 P<0,01	39,1±1,4 P=0,001	26,2±0,9 P>0,2	14,1±0,6 P<0,05
Argon-oxygen medium	42,3±0,8 P>0,9	32,8±0,8 P<0,01	26,6±0,9 P<0,001	20,9±0,5 P<0,001	13,4±0,3 P<0,001

*Translator's Note: Commas indicate decimal points.

Results of the study of body temperature for the animals during the experiments are presented in Table 2.

TABLE 2. CHANGE OF BODY TEMPERATURE FOR RATS IN HERMETICALLY-CLOSED CHAMBER (MEAN DATA).

Composition of gas medium	Temperature of gas medium, °C				
	22	27	31	36	40
Air	-3.8*	-1.6	+0.6	+2.9	+2.5
Helium-oxygen medium	-8.2	-4.6	-1.0	+2.2	+2.1
Argon-oxygen medium	-3.1	-0.4	+1.2	+2.2	+2.0

*Minus signifies reduction, a plus sign indicates an increase of body temperature.

As can be seen from the table, the replacement of nitrogen in the air by argon in the atmosphere of the hermetically-enclosed chamber and a temperature of 22° did not reflect on the longevity of the animals. In addition the lack of

[5]In this table data are given obtained in the previous work under conditions of air and helium-oxygen media for comparison (Dianov, 1967).

a substantial difference in the degree of reduction of body temperature for the animals in the air and argon-oxygen media was very important (Table 2 and Figure 2). At the same time the duration of the animals and the reduction of the body temperature were much greater in the experiment conducted using helium in place of nitrogen than in the air and argon-oxygen media. These results of the investigation confirm the presence of a direct relationship between the level of hypothermy and the duration of animals in hermetically sealed chambers. Thus, the experimental data obtained confirm the hypothesis that an increase in duration of animals in a helium-oxygen medium is related to the more pronounced reduction of body temperature than in the air which is conditioned primarily by the high thermal conductivity of the helium.

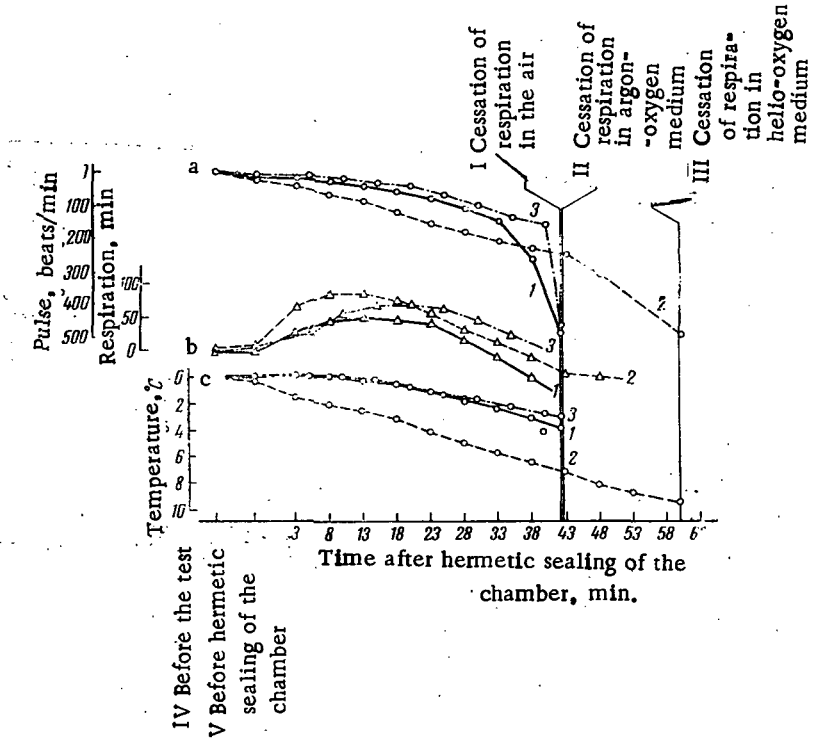

Figure 2. Pulse Frequency (a), Respiration (b) and Body Temperature (c) in Animals with Temperature in the Chamber of 22° (Comparison with Initial Values). 1, Air; 2, Helium-oxygen medium; 3, Argon--oxygen medium.

From the data presented in Table 1 it is also evident that during an increase of the surrounding temperature above 22° replacement of the atmosphere nitrogen by argon in the atmosphere of the hermetically-sealed chamber leads to reduction in longevity of the animal. The reasons for this reduction have not yet been sufficiently studied. Analysis of the changes in the rate of respiration and rate of heat contractions in the rat in the experiment which we conducted did not provide any additional information which would permit us to form a hypothesis to explain the obtained facts. /14

Thus, in our study we determined that replacing the atmospheric nitrogen with argon in the atmosphere of the hermetically-sealed chamber at a temperature of 22° did not affect the longevity of the animals which were under conditions of oxygen deficiency and increased concnetnrations of carbon dioxide. The experimental data obtained confirmed the supposition that an increase in longevity of animals in a hermetically-sealed chamber with a helium-oxygen medium at a temperature of 22° is related to the high thermal conductivity of helium.

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POSSIBILITY OF USING ADAPTATION OF HYPOXIC HYPOXIA IN A SYSTEM OF CONDITIONING

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P. V. Buyanov, V. S. Formin and D. Yu. Arkhangel'skiy

ABSTRACT. The possibility of conditioning the temperature and pressure testing chamber in order to increase human resistance to certain extreme factors were studied. Three series of investigations were conducted: the first at an "altitude of 4,000 m with optimum air temperature, the second at an "altitude of 4,000 m with high air temperature (50°) and the third at an "altitude" of 2,000 to 5,000 m with changing physical stress at an optimum air temperature. Length of the conditioning cycle was twenty days. Results of the investigation showed that the third series of conditioning, planned with consideration of the basic principles of athletics, was the most effective for increasing the resistance to extreme factors (to high temperature, to transversely oriented stresses, to vestibular-optical-kinetic stimuli).

There are a sufficient number of works devoted to the study of the change /148
of the resistance of an organism to the action of different factors (intoxication, infectious diseases, penetrating radiation, cold, etc.) due to the effect of adaptation to hypoxic hypoxia. There are indications that hypoxia has a wide spectrum of action on the organism, which means that adaptation to it provides an increase of functional possibilities and improvement of the ability to withstand many external influences. In this connection there were many suggestions as to the effectiveness of using high-altitude hiking and mountain climbing in a training program (Parin, Agadzhanyan et al., 1966). There have been many investigations of the changes in the capacity of man to withstand different extreme factors due to staying in the mountains or in pressure chamber conditioning (Vasil'yev, Kotovskaya, 1965; Malkin, Asyamolova, Kochetov, 1965, and others). Encouraging results have been obtained. However, a number of problems have still not been finally solved.

In particular, the effect of adaptation to hypoxia and a capacity to withstand high temperature has not been sufficiently studied. F.T. Agarkov (1959), and also V. B. Malkin et al., (1968) discovered an increase in

the resistance of white rats to overheating after adaptation to hypoxia. In human beings, according to the data of V. B. Malkin, G. D. Yukhnovskiy and S. S. Markaryan (1968), resistance to high temperature was not increased after a stay in high-mountain conditions, but sometimes it even worsened.

In addition there are still not sufficient data on the significance of the overall influence of hypoxia and muscular work for consequent capacity of man to withstand certain extreme factors.

In this work a study of these problems was attempted in order to use /149
the results for increasing the resistance of an organism to extreme influences.

Three programs of training were evaluated, each of which included sixty-minute influences of different factors daily for twenty days. The investigations were conducted in a temperature and pressure testing chamber with twelve test subjects participating: six persons participated in the first training program, three in the second and six in the third (certain test subjects participated in two series of investigations with intervals between the investigations of no less than six months).

The training programs included:

I Program — combination of constant altitude (4,000 m) and muscular load (300 kgm/min);

II Program — combination of constant elevation (4,000 m), muscular load (300 kgm/min) and high temperature (+50°);

III Program — combination of elevation, changing every few days from 2,000 to 5,000 m, and muscular load, which was from 315 to 440 kgm/min on different days (Figure 1).

As a muscular load cyclical raising of a load was selected using an immovable block at the rate of 30 times per minute. The work was accomplished in five-minute cycles with an interval of five minutes between them. In experiments of the III series during conditioning at an elevation of 3,000 m and higher the test subjects accomplished the five minute work each time only at an intermediate elevation of 2,000 m (immediately before lifting to the given height). This was a kind of warmup before the principal load.

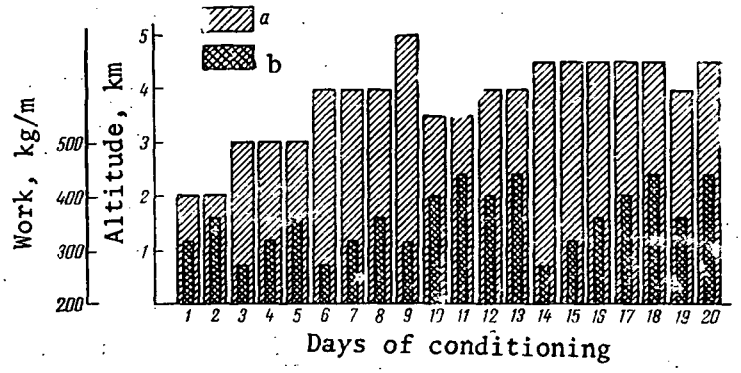

Figure 1. "Altitude" (a) and Value of Physical Load (b) by Days of Conditioning in the III Series of Tests.

To determine the efficacy of the conditioning, before the beginning and after the and of each cycle of conditioning, we checked the resistance of the test subjects to transverse stresses, to /150 hypoxia (according to time of stay at elevation of 7,000 meters), to high temperature (+60°) and to complex stimulation of the vestibular, visual and motor analyzers (with evaluation of the efficiency and physiological reactions of the operators). In addition we studied reactions to functional loads (squatting test, stop tests and orthostatic tests).

The ability to withstand transverse stresses on a centrifuge with a radius of 7.25 m was determined for the maximally achieved value during continuous increase of the g-forces with the gradients of 0.1 units/sec. Previously we had conducted a familiarization-warmup rotation with an overload of 4 units lasting for 1.5 minutes, and after a ten-fifteen minute rest we carried out the principal rotation. In all of the cases the total vector of the g-force was 78° to the longitudinal axis of the body. The criterion for achieving the g-force load limit was an index of relative bradycardia — OKIPS (Arkhangel'skiy, Babushkin, Marukhanyan, 1966), which enabled us to determine the maximally tolerable values of g-forces at which pathological changes do not develop in the organism (Petrukhin, Stepantsov, Marukhanyan, 1967).

In order to insure comparability of the results our investigations were conducted after ending the cycle of conditioning at constant time periods: the heat tests and functional tests after one-two days, the hypoxic test after four--eight days, the vestibular test and centrifuge tests after eight-fifteen days.

Comparison of the results of the heat test were the most significant. It turned out that after conditioning in the first and second programs the time for withstanding high temperature did not change and even decreased. We noticed improvement in durability in only two instances.

Simultaneously we noticed a slight increase in the buildup of heat in the organism (Figure 2). On the other hand after the III series of conditionings we observed a clear tendency to improvement of the ability to withstand high temperature (by 17% on the average in time). Weight losses due to elimination /151
of perspiration and buildup of heat in the organism decreased noticeably. It is interesting that after the III series heat production was also reduced (by 0.2 kcal/min on the average), while heat production increased somewhat after the first two series.

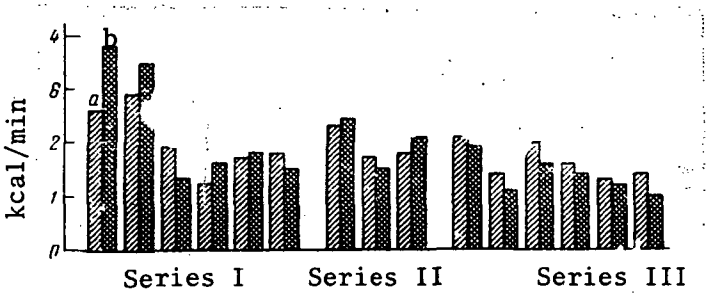

Figure 2. Accumulation of Heat in the Organism of the Test Subjects With Temperature of the Surrounding Environment of +60°. a, Before conditioning; b, After training.

It is possible that adaptation to hypoxia promotes adjustment of tissue metabolism. The more complete the adjustment, the less the heat production and the more improved the ability to withstand thermal stress. It should be recalled that during thermal adaptation it is mainly the mechanisms of heat transfer (perspiration elimination, vascular reactions) which are improved.

After these training periods resistance to other factors also increased, especially to hypoxia (by 25% based on time spent at an elevation of 7,000 m). However, in contrast to the heat tests, we did not observe a significant difference between the different series. Evidently, this is explained by the fact that certain tests were conducted after relatively long intervals, up to eight days, and also by the fact that during conditioning we did not consider the

individual peculiarities of the test subjects. Nevertheless the results obtained are encouraging. Above all, the data are confirmation of the fact that adaptation to hypoxia in combination with muscular work in general increases the functional possibilities of an organism. Thus, in trials with stop-tests and squatting after conditioning periods we observed a smaller change of blood pressure and pulse rate, and also a quicker regeneration of the initial level of these indices. The orthostatic trials did not turn out to be significant, because the ability to endure it was high even before the training.

On the other hand, improvement of the ability to withstand stresses, and also the increase in resistance and improvement of the operator activity under conditions of prolonged (up to twenty minutes) multiple stimulation of the vestibular, visual and motor analyzers in certain test subjects shows that with proper organization of training periods a markedly positive effect can be achieved. In this plan, in our opinion, training under conditions of daily changing values of hypoxia and physical load, as is the case in the III series is the most effective. The II program of conditioning is the least convenient although positive results for a number of tests were attained after it. Analysis of data of functional trials revealed a favorable effect of the conditioning on hemodynamic parameters.

From the table it is evident that after this series of conditionings the pulse and minute volume of the blood decreases even at a state of rest, the rate of propagation of a pulse wave decreases and the peripheral resistance increases.

Thus, our investigation showed that the simultaneous action of hypoxia and /152
muscular work induces the development of adaptation shifts in the organism, which promote improved ability to withstand not only hypoxic hypoxia but also high temperature and to some degree, statokinetic stimulation and transverse stresses. These shifts in the conditioning effect are most pronounced during the changing nature of the influence (III series of conditioning), which again corroborates the necessity of considering the basic principles of conditioning as developed by the medical aspects of athletics in the compilation of programs and methodologies. As a result of the repeated action of hypoxic hypoxia and physical

stress of differing levels, it is obvious that the mechanism for regulation which took shape is adequate not only for these stimuli but also for the action of other factors. Thus, changes which arise after conditioning at high temperature and which are aimed at the preservation of the heat balance are indicative of development of adaptation-trophic adjustment at the tissue level. This is confirmed by the smaller amount of heat production in the organism during tests following conditioning, which is especially important due to the reduction of total heat load on an organism and consequently the increase in the amount of time high temperatures can be endured. The monotonous influence often has no effect. Including the systematic influence of high temperature in the conditioning program reduces the resistance to certain influences, evidently due to the reduction in the vascular tone.

TABLE. CHANGE IN HEMODYNAMIC INDICES UNDER CONDITIONS OF REST BEFORE AND AFTER CONDITIONING (MEAN DATA).*

Indices	I Series			II Series			III Series		
	Before	After	Difference, %	Before	After	Difference, %	Before	After	Difference, %
Rate of heart contractions, beats/min	61	59	−3,0	59	61	+3,4	57	59	+3,6
Pulsating vol, ml	104	100	−3,7	104,2	77,3	−25,9	98,8	100,8	+3,0
Minute vol., l	6,33	5,9	−6,6	6,16	4,71	−23,6	5,64	5,94	+5,2
Specific peripheral resistance, arb.u.	24,4	26,0	+6,5	24,5	33,7	+36,3	26,0	25,8	−0,7
Rate of propagation of a pulse wave, cm	678	722	+6,2	701	648	−7,6	690	739	+7,2
Rate of propagation of a pulse wave along muscle vessels in relation to elastic vessels	1,06	1,11	+4,7	1,09	1,0	−8,2	1,11	1,11	0

* Translators' Note: Commas indicate decimal points.

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IV. TOXICOLOGY

EFFECT OF CERTAIN GASEOUS TOXIC SUBSTANCES ON THE RESISTANCE OF ANIMALS TO ACUTE HYPOXIC HYPOXIA

B. I. Abidin, N. M. Asyamolova and A. K. Sgibnev

ABSTRACT. In this article the problem of the effect of different concentrations of a mixture of gaseous chemical substances on the ability of animals to withstand acute hypoxic hypoxia is examined. Comparative data of the change in EEGs. EKGs and respiration of inoculated and intact rats under conditions of rarefied atmosphere, which corresponds to an elevation of 10,000 m. Gaseous chemical substances change the relationship of the organism of animals to the influence of hypoxic hypoxia, and reduce the "physiological ceiling" and increase the ability to withstand acute oxygen deficiency.

The atmosphere of the cabins of space vehicles is a complex gas mixture in /154
which there is a large amount of gaseous chemical substances in addition to oxygen and carbon dioxide. Varied chemical compounds may get into the air of a cabin in the process of the vital activity of man, in the use of polymer materials and on-board equipment. While conducting experiments lasting many days, almost seventy different chemical substances were detected in the atmosphere of hermetically sealed mockups of the cabins of space vehicles (Adams et al., 1966). According to the data of T. Veber (1967), there are almost 400 compounds which represent twenty-two chemical groups, emanating from man alone.

Thus, under flight conditions, man may come in contact with a large group of chemical substances which might change the relationship of his organism to other spaceflight factors. It is known that inhalation of carbon monoxide before beginning of irradiation increases the resistance of animals to ionizing radiation (Tiunov, Kustov, 1969; Konnecci et al., 1955; Bonet-Maury, Patti, 1954). Nitrogen and inert gases at increased atmospheric pressure also have a radioprotective effect (Paribok, 1962).

Oxygen deficiency is an essential element in the mechanism of the action on the organism of many factors which are characteristic of flight: changing

weightlessness, high and low temperature and a large number of other factors. In this connection the study of the relationship of animals which have been poisoned with a complex gas mixture to the influence of acute hypoxic hypoxia is of great interest, and is one of the real factors of spaceflight. /155

The complex gas-air mixture which was obtained as a result of the final thermo-oxidative destruction of a snythetic polymer based on pentaerythritol and fatty acids contained gaseous products belonging to different classes of compounds: complex esters, aldehydes, ketones, organic acids, alcohols, carbon monoxide and several other substances. Inoculation of the animals was carried out in a 100-ℓ hermetically sealed chamber using the static method with exposure time of one hour.

Ten white rats were subjected to inoculation with products of the thermal destruction of a polymer in the amount of 1,200 mg. Such an amount of a decomposed product caused death in approximately 50% of the animals in one-to-three days following inoculation. Before and immediately following the inoculations we determined the O_2 consumption for the rats (Figure 1).

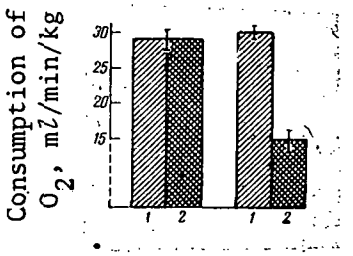

Figure 1. Change in the Consumption of Oxygen in Rats When Affected by Products of Thermal Destruction of a Polymer in the Amount of 1,200 mg. 1, Control; 2, Test.

From the data presented in the figure, it is evident that well before inoculation the consumption of oxygen in the experimental animals was the same as in the control animals, comprising, on the average, 29.0 ± 1.4 mℓ/min/kg, while after the inoculation this figure decreased by half, becoming 14.7 ± 1.6 mℓ/min/kg. Following this the animals were placed in a pressure chamber and subjected to the action of acute hypoxic hypoxia. The "ascent" was accomplished at a velocity of 40 m/sec to the "elevation" of 12,000 m. During the "ascent" we observed the behavior and general condition of the animals and recorded time at which they perished (Figure 2). As can be seen from the drawing all ten control

animals perished at an "elevation" of 12,000 m within nine minutes, while three of the ten experimental rats did not perish even after a twenty-minute stay under conditions of rarefied atmosphere.

In order to better understand the condition of the adaptative reactions to acute hypoxic hypoxia in the animals which were inoculated with products of thermal destruction of the polymer we set up four series of rat experiments with recording of electroencephalograms, electrocardiograms and respiration.

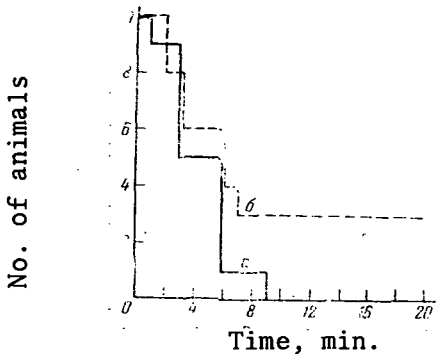

Figure 2. Longevity of Rats Who Have Been Subjected to Inoculation with Products of the Thermal Destruction of a Polymer (1,200 mg) and Intact Animals Under Conditions of Rarefied Atmosphere, H = 12,000 m. a, Control; b, Test.

In series I we did the inoculations with thermal destruction products which were formed during the decomposition of 2,700 mg of a polymer. Such an amount of a decomposed polymer caused death to the experimental animals during the first days.

We conducted thirteen experiments. /156 In two tests the "ascent" was made to an "elevation" of 12,000 m, in eleven tests it was to 10,000 m. In their external appearance the inoculated animals differed sharply from the intact animals. They were sluggish, their fur was dull and dishevelled. The temperature in the rectum of the inoculated rats was on the average 3° lower than for the control rats. Differences in the recorded physiological functions appeared even before the beginning of the influence of the rarefied atmosphere in the inoculated and intact animals. These differences are even more clearly evident on the electrocardiogram. The rate of heart contractions for the inoculated animals was 240-300 beats/min. The shape of the EKG peaks was altered: the R peak was frequently decreased, the S-T was shifted upward, the P-Q was lengthened. The latter confirmed disorder of the atrioventricular conductivity. In some of the inoculated animal studies the sinus rhythm was unstable and there were signs of atrioventricular blockade. In the control rats the rate of heart contractions was 400-600 beats/min, a

stable sinus rhythm. The slight increase in the heart rate in the control animals is evidently a reaction to being held down.

For respiration the changes are less constant, but nevertheless respiration was curtailed in the majority of the inoculated animals. Normally there was an increase in the number of high-frequency fluctuations on the electroencephalogram with some reduction in height. The characteristic changes in the recorded physiological indices after inoculation are presented in Figure 3. The EEG of an intact rat shows wave groups with frequency of 9-10 Hz and amplitude of 50 μV, on which high-frequency fluctuations are superimposed, small wave groups of 5-6 Hz and up to 60 μV and single waves of 3-4 Hz and 80 μV. High-frequency /157
fluctuations with amplitude of 30 to 50 μV, single fluctuations of 3-4 Hz and small wave groups of 5-6 Hz and amplitude of 30 μV predominated on the EEG of an inoculated rat. The EKG of an inoculated rat showed a heart rate of 153 per minute (in the control it was 564 per min). The ventricular rhythm and the S-T interval were shifted significantly higher than the isoline. The respiration rate for the inoculated rats was 36 per minute (132 per minute for the control).

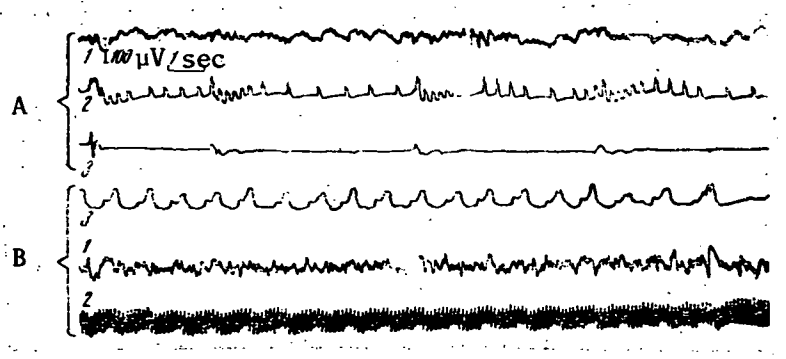

Figure 3. Physiological Indices for a Rat Inoculated With Products of Thermal Destruction, 2,700 mg of Polymer (A) and a Control (B). 1, EEG; 2, EKG; 3, Respiration.

Thus, we noticed clear disturbances on the part of the recorded physiological indices after the action of a mixture of toxic substances in rats.

In all of the tests, we detected a higher sensitivity to acute hypoxic hypoxia in the inoculated rats as the rarefaction increased in the chamber, i.e., the so-called "physiological ceiling" of the rats was reduced significantly. The increase of motor activity, the disturbance to heart activity, respiration and the central nervous system due to the action of oxygen deficiency in three of the inoculated animals was observed beginning at an "elevation" of 3,000 m, in five of them at an elevation of 4,000 m and at an elevation of 5,000 m in three of them. At the same time similar changes developed only during rarefaction corresponding to an elevation of 8,000-10,000 m in the control animals. Recordings on the EEG, EKG and respiration for the inoculated and intact rats are presented in Figure 4. Section A has recordings before the "ascent": shortening of the rate of heart contractions, the rate of respiration in an inoculated rat and prevalence of high frequency fluctuations are clearly shown. At an "elevation" of 4,000 m (Section B) tonic and clonic spasms developed in the inoculated rat, heart activity was disturbed, bradycardia of 120 beats per minute appeared, and the rhythm became unsteady. In this connection the shape of the peaks of the ventricular set was sharply changed: the S-T interval was shifted upwards so that at times the R and T peaks came together, forming monophase oscillations. Single and group polytotic ventricular extrasystoles appeared. The respiration became uneven in rate and amplitude. A slow rhythm predominated on the electroencephalogram, with the amplitude increasing to 120-200 μV on the average. Later (in the given example in the eighteenth minute after achieving the "elevation" of 10,000 m) partial normalization of the recorded functions began in the inoculated animals. A sinus rhythm with frequency of 240 beats per minute appeared. The high-frequency oscillations (Section C) again prevailed on the EEG. Such a phenomenon was observed during the "ascent" in the majority of the inoculated animals. The control rat perished at this time. Terminal respiration began and heart activity was abruptly disturbed (complete atrioventricular blockade and distortion of the EKG peaks). There was pronounced depression of the biocurrents on the electroencephalogram. /159

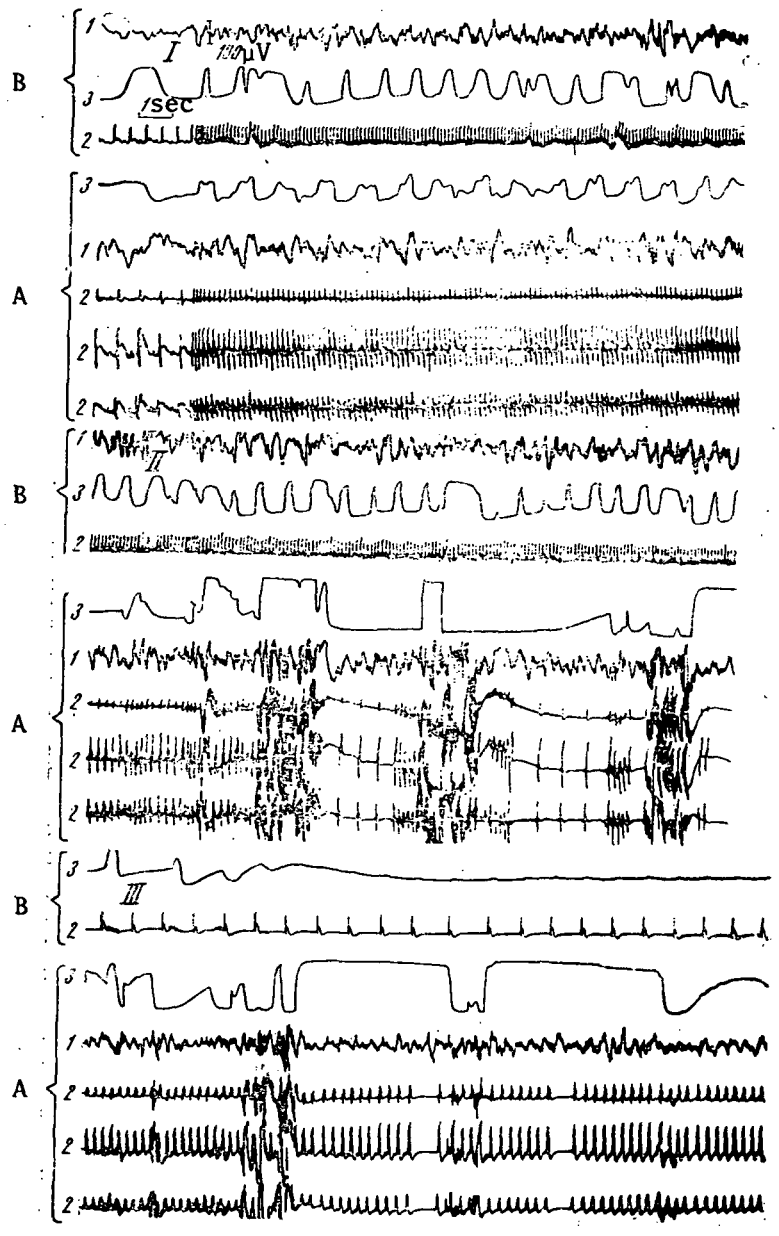

Figure 4. Physiological Indices for a Rat Inoculated with Products of Thermal Destruction, 2,700 mg of a Polymer (A), in Comparison with an Intact Rat (B) During "Ascent" Up to an "Elevation" of 10,000 m. 1, Before ascent; 2, 4,000 m; 3, 10,000 m, 18th minute. Remaining designations are the same as in Figure 3. The EKG is given in three standard leads.

Thus, we noticed an increased sensitivity to oxygen deficiency in the inoculated animals, which was expressed in an earlier manifestation of functional disorders associated with hypoxia. However, the survival rate for the inoculated animals was higher than for the control animals (see table).

TABLE. LONGEVITY (IN MIN) OF INTACT RATS AND RATS INOCULATED WITH PRODUCTS OF THERMAL DECOMPOSITION OF A POLYMER UNDER CONDITIONS OF A RAREFIED ATMOSPHERE (H = 10,000 m).

No. of tests	Amount of decomposed matter, mg							
	2,700		900		200		100	
	Control	Inocul.	Control	Inocul.	Control	Inocul.	Control	Inocul.
1	2 min 30 sec	1 min 30 sec	9	30	16	11	8	2
2	8 min	77	2	7	26	70	11	11
3	30 sec							
3	30	45	31	26	44	49	2	2
4	10	40	49	55	8	2	11	12
5	2	25	171	177	7 min 30 sec	93	21	26
6	13	30	8	3	1 min 10 sec	58	2	11
7	18	26	7	90	4	8	23	10
8	6	23	34	56	3 min 30 sec	5	1	7
9	3	15	161	163	14	7	14	8
10	5	15	5	16	4	8	12	20
11	7	15	13	26	18	3	3	4
12			7	32	45	25		
13			157	162				
Reliability of diff.		Incr. $R<0.01$		Incr. $R<0.05$		$R>0.05$		

In the II series of tests the amount of decomposed polymer was 900 mg. We conducted fourteen experiments. Of these fourteen, in one test the rarefaction in the chamber corresponded to 12,000 m, in thirteen tests it corresponded to 10,000 m. We also noticed differences in the recordings of the physiological functions for animals which had been subjected to inoculation in comparison to the intact animals in this series of experiments, although they were expressed to a lesser degree than in the previous series. The sinus rhythm was preserved

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in the electroencephalogram for the inoculated rats and we noticed only a moderate retardation of the rate of heart contraction. There were individual ventricular extrasystoles in some of the animals. The heart beat rate in the inoculated animals was within the limits of 320-450 beats/min. The respiration rate in the inoculated animals was also somewhat reduced and did not exceed 36 per minute, and in the control it did not exceed 60 per minute. On the electrocorticogram we noticed a predominance of high-frequency oscillations with a reduction of their amplitude, which is a phenomenon of desynchronization. For illustration purposes Figure 5 shows a recording of EEG, EKG and respiration of an inoculated and a control animal which was made before the action of the rarefied atmosphere and one hour after inoculation with the mixture of toxic substances. Changes in the physiological indices due to the influence of the hypoxic hypoxia in the inoculated animals in this series of tests developed somewhat later (that is, at a higher "elevation") than in the preceding series However, as in series I of the tests, the hypoxic disorders began earlier in comparison with the control animals. In three of the animals which had been inoculated, phenomena of oxygen deficiency were revealed by the recorded indices beginning at an "elevation" of 5,000-6,000 m, and in eleven of them beginning at an elevation of 8,000 m. As in the preceding series, there were statistically reliable differences of survival rate for the inoculated and intact animals in the given experiment (see the table).

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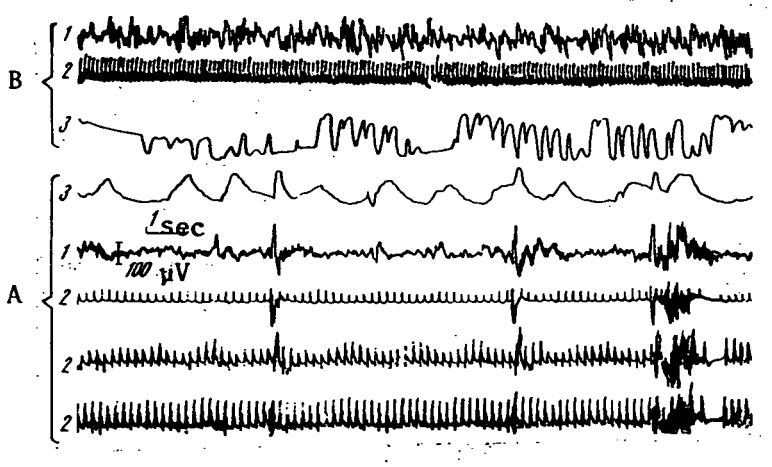

Figure 5. Physiological Indices of a Rat Inoculated with Products of Thermal Destruction, 900 mg of a Polymer (A) and a Control (B). Designations are the same as in Figures 3 and 4.

In series III of the tests 200 mg of a synthetic polymer were subjected to thermal decomposition. In the twelve experiments of this series we were not able to notice abrupt changes in the recorded indices for the animals after their inoculations. The heart beat rate for the inoculated rats was less frequent in five of the experiments, more frequent in five, and the same as for the control animals in two experiments. But the shape of the EKG peaks still remained changed: we noticed some shifting of the S-T interval above the isoelectric line, and in individual tests we noticed an increase of the Q peak and a reduction of the R peak. There were no reliable differences for respiration, although in a majority of the tests it was less frequent than for the inoculated animals. A predominance of high-frequency oscillations remained on the electroencephalogram for the inoculated animals. For illustration purposes Figure 6 /162
presents recordings of the physiological functions of a rat one hour after inoculation with 200 g of decomposition products of a substance in comparison with the intact animals. It is clearly evident in the oscillograms that the degree of changes in the inoculated rats in the present variant of the test is significantly less than for the preceding series. The phase of hypoxic changes in the control and experimental rats occurred almost simultaneously at an "elevation" of 8,000-9,000 m. The difference in survival rate time disappeared: the inoculated animals survived in seven experiments and the control animals survived in five experiments (see table).

In series IV we conducted eleven experiments. The amount of decomposed polymer was 100 mg. In this case we were not able to notice substantial changes in the behavior of the rats or the nature of the above-described changes on the oscillograms for the experimental and the control animals. In Figure 7, I, we have given recordings of the EKG EEG and respiration of an inoculated and an intact rat before the action of acute hypoxic hypoxia. As can be seen from the curves, the rate of heartbeats and respiration is practically identical in them, less than can be distinguished by the EEG.

The hypoxic disorders on the EKG for all of the inoculated and control animals began nearly simultaneously (Figure 7, III). Disturbances of respiration and EEG in some of the inoculated animals began somewhat later, which is shown clearly in Figure 7, III. In spite of this the survival rate time at the

"elevation" for the inoculated and control rats is approximately the same (see table). In five experiments the control rats survived, and in four of them the inoculated rats survived, while in two of them they perished simultaneously.

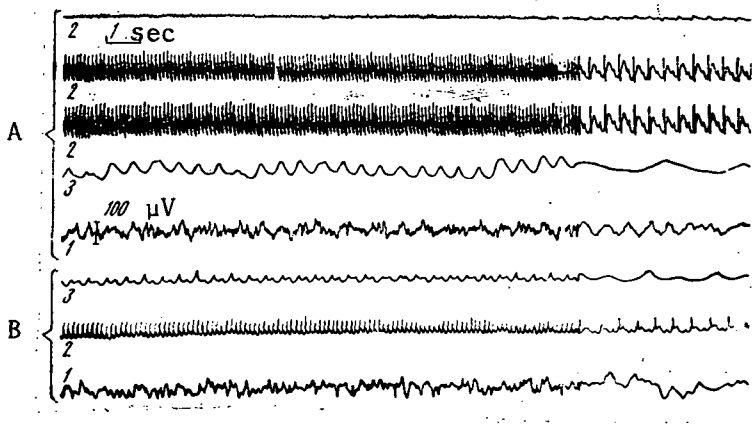

Figure 6. Physiological Indices of a Rat, Poisoned With Products of the Thermal Destruction of 900 Mg. of Polymer (A) and A Control Rat (B). Symbols same as in Figure 3.

Thus, products of the thermal decomposition of the polymer in an amount of 2,700-900 mg not only caused gross disturbances in the animal's circulation, respiration and nervous system, but they evidently led to an engagement of third-order adaptative reaction (Barbashova, 1960) and changeover to anaerobic type respiration. This obviously explains the higher survival rate of the inoculated animals under conditions of rarefied atmosphere in comparison with the intact animals. The products of thermal decomposition of a polymer in the amount of 200 mg also caused disorders of circulation, respiration and nervous system. However, the degree of their manifestation was less and the organism evidently was not able to prepare itself for engagement of sufficiently strong tissue adaptative reactions in response to the subsequent acute hypoxic hypoxia. After the action of products of thermal destruction of 100 mg of a polymer we detected no changes in the physiological functions under investigation./163 However, changes of respiration and the nervous system in response to acute hypoxic hypoxia in some of the inoculated animals came somewhat later than for the control animals. This indicates an initial adaptative reaction to hypoxia in individual animals which have been inoculated by a mixture of chemical agents in a relatively small concentration.

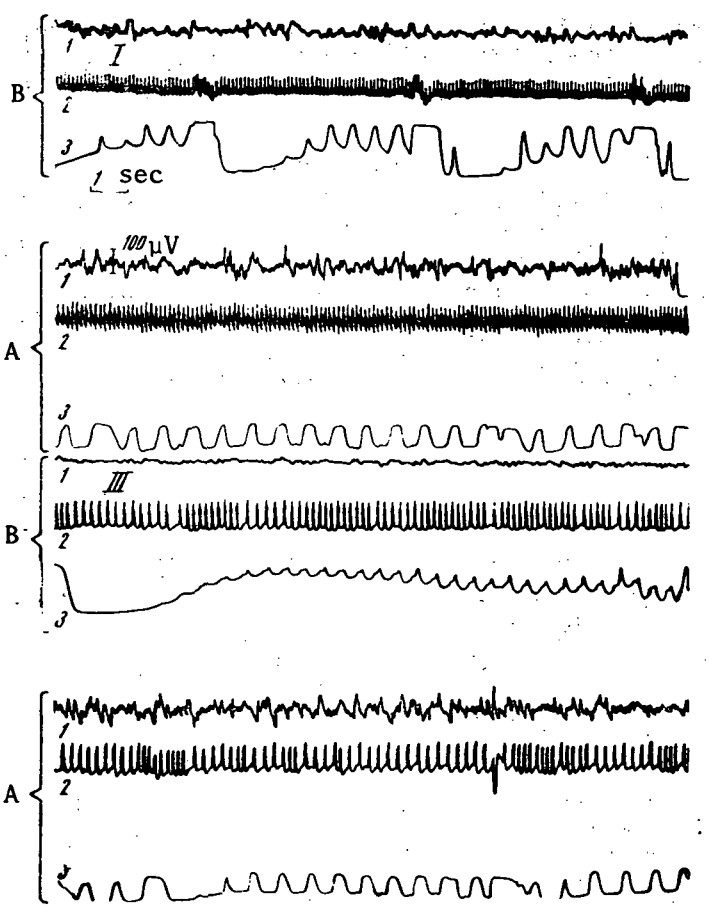

Figure 7. Physiological Indices for a Rat Inoculated with Products of Thermal Destruction of 100 mg of a Polymer (A), and a Control Rat (B) During Ascent to an Elevation of 10,000 m (Third Minute). Designations are the same as in Figure 3.

Thus we were able to establish that chemical substances can change the relationship of a living organism to the influence of acute hypoxic hypoxia, reducing the "physiological ceiling" and increasing its ability to withstand acute oxygen deficiency.

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CERTAIN PECULIARITIES OF THE BIOLOGICAL ACTION OF GASEOUS TOXIC SUBSTANCES WHICH ARE DISCHARGED INTO THE ATMOSPHERE FROM THE URINE AND FECES

V. V. Kustov, V. I. Mikhaylov and L. T. Poddubnaya

ABSTRACT. In tests on white rats it was determined that a variety of gaseous toxic substances which enter the air from fresh and stored excrement has not only an irritating but also a generally toxic action.

The literature gives numerous examples of the harmful action of gaseous malodorous emissions from latrines (Erisman, 1875, 1901; Korenchevskiy, 1909; Flyuri and F. Tsernik, 1938), feces and urine (Clemedson, 1959; Slager, 1962) on an organism. /164

However, experimental study of the toxic action of a number of gaseous discharges from excrement has not been conducted. Investigations of such a nature are necessary in order to evaluate the dangers of gas discharges from sanitation devices placed in hermetically-enclosed spaces with small volume. The present work is devoted to the study of that problem.

We conducted tests on white male rats weighing 20-22 g. We inoculated the animals with gas discharges from urine and feces (separately) by the dynamic method in hermetically sealed glass desiccators for two hours.

With this goal in mind the room air, which had been previously purified in columns which were filled with pumice containing sulfuric acid, alkali, hopcalite and silica gel, was drawn at a speed of 0.5 l/min by the aid of an air blower through a "flute" containing urine or a hermetically sealed vessel with collected accumulations of feces and then entered the inoculating chamber. /165

During inoculation we observed the general condition and behavior of the experimental animals. Before the beginning of the experiment and after its completion we determined the cholinesterase activity of the blood in the white mice by the A. A. Pokrovskiy (1961) method; the blood catalase by the A. N. Bakh and S. R. Zubkova method (1950), the amount of carboxyhemoglobin in the /166

blood by Vol'f method (Teyfinger et al., 1959) and the amount of acetylcholine according to the method of Khestrin using the L. Ya. Livshits and V. I. Rubin modification (1961); and we recorded the value of the latent period of the reflex in response to an electric current shock.

The experiments which we conducted showed that the entrance of the collection of gaseous toxic substances from fresh urine into the chamber (Table 1) caused motor stimulation, unrest and an increase in the respiration rate of the animals during the first thirty to thirty-five minutes of the test. The white mice closed their eyes and wiped their snouts with their paws. Approximately one hour after the beginning of the inoculation the animals settled down, sat in groups and hid their snouts in each others' fur.

During the examination of the inoculated mice we noticed no substantial shifts in the amount of carboxyhemoglobin in the blood and its catalase activity (Figure 1). We did establish a noticeable increase in the amount of acetylcholine in the blood and an increase in the cholinesterase activity of the blood (Figure 2).

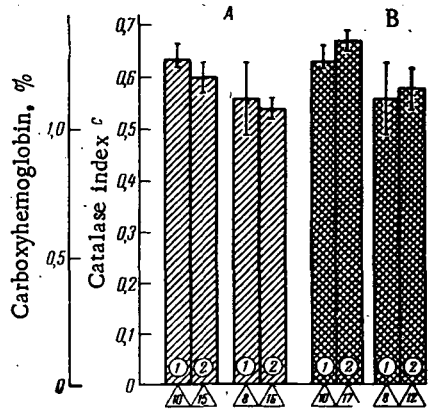

Figure 1. Effect of a Group of Gaseous Toxic Substances Discharged from Fresh (A) and Stored (B) Urine, on the Amount of Carboxyhemoglobin and the Catalase Activity of the Blood of White Mice. 1, Control; 2, Test; in the triangle, Number of animals.

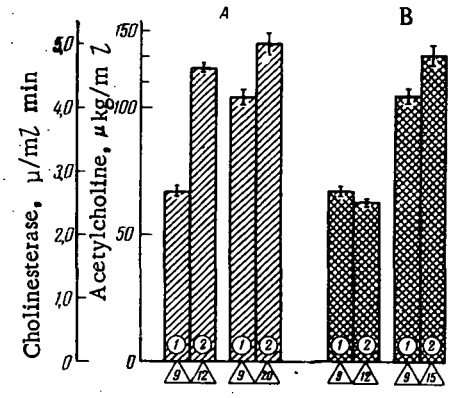

Figure 2. Effect of a Group of Gaseous Toxic Substances Discharged from Fresh (A) and Stored (B) Urine on the Cholinesterase Activity and the amount of Acetylcholine in the Blood of White Mice. Designations are the same as for Figure 1.

TABLE 1. AMOUNT OF GASEOUS TOXIC SUBSTANCES IN THE AIR OF AN INOCULATION CHAMBER AFTER ITS DIFFUSION THROUGH URINE.

Substance	Concentration, mg/l		Substance	Concentration, mg/l	
	10-day urine	Fresh urine		10-day urine	Fresh urine
Aldehydes	Traces	Traces	Oxides of nitrogen in conversion to N_2O_5	0.002	0.0007
Ammonia and aliphatic amines	0.0008	Traces	Carbon monoxide	0.003	0.012
Ketones	0.0004	0.007	Sulfur dioxide	0.00006	Not pres.
Organic acids in conversion to CH_3COOH	0.052	0.009	Hydrocarbons in conversion to C	0.043	0.042
Indole	Not pres.	Not pres.	Phenols	0.0007	0.0006
Mercaptans and hydrogen sulfide	Traces	---	Oxygen	20%	20%
			Carbon Dioxide	1%	0.9%

The action of a group of gaseous toxic agents discharged by urine stored for ten days (see Table 1) caused the same intoxication effects on the experimental animals as during tests in which air is passed through fresh urine. The level of the carboxyhemoglobin in the blood and its catalase activity practically did not change (Figure 1) upon completion of inoculation of the white mice. The amount of acetylcholine in the blood of the experimental animals increased; however, its cholinesterase activity did not change at this time (Figure 2).

The clinical picture of poisoning by gaseous discharges from fresh and /167
stored feces was practically identical. During the first twenty-five to thirty minutes of inoculation it fully resembled the symptoms of poisoning by irritating poisons. The experimental animals were stimulated: they continuously /168
moved around the chamber, made high jumps, their respiration noticeably increased; the animals rubbed their snouts with their paws and closed their

eyes. Approximately one hour after the beginning of the inoculation the mice settled down, sat around in groups, and hid their snouts in each others' fur.

At the completion of inoculation by discharges from fresh feces (Table 2) the level of the carboxyhemoglobin in the blood of the experimental animals reached ~ 1.2% (Figure 3). The catalase activity (Figure 3) and the cholinesterase activity of the blood (Figure 4) practically did not change, but the amount of acetylcholine definitely increased (Figure 4).

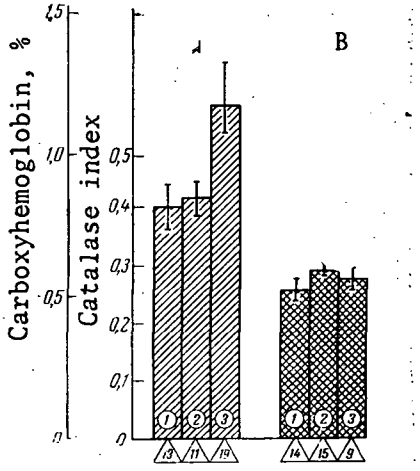

Figure 3. Effect of a Group of Gaseous Toxic Substances, Discharged by Fresh Feces, on the Level of Carboxyhemoglobin (A) and the Catalase Activity (B) of the Blood of White Mice. 1, Intact animals; 2, Control; 3, Test; In the triangle: Number of animals.

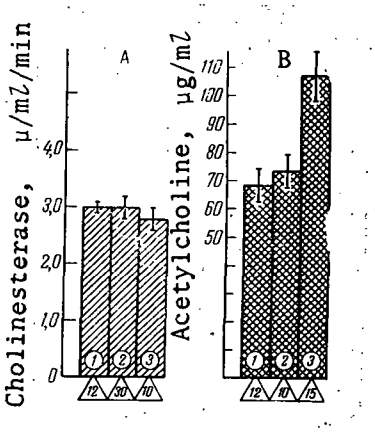

Figure 4. Effect of a Group of Gaseous Toxic Substances, Discharged by Fresh Feces, on the Cholinesterase Activity and the Amount of Acetylcholine in the Blood of White Mice. Designations are the same as in Figure 3.

Disturbance of the ratio between the cholinesterase and acetylcholine evidently promoted some accumulation of the latter in the organism, which intensified the process of inhibition in the nervous system, which was expressed in an increase in the time of the latent period of the flexor reflex of the animals (Figure 5).

We noticed an increase in the level of the carboxyhemoglobin in the blood and a certain tendency towards an increase in the amount of acetylcholine

(Figure 6) in the white mice which had undergone poisoning by gaseous discharges from stored fecal masses (Table 2). The cholinesterase activity of the blood decreased significantly, and the catalase activity practically did not change (Figure 7).

TABLE 2. AMOUNT OF GASEOUS TOXIC SUBSTANCES IN THE AIR OF A CHAMBER AFTER PASSAGE THROUGH A CONTAINER WITH FECES.

	Amt. of substances in the air above feces, mg/l			Amt. of substances in the air above feces, mg/l	
	Fresh	Stored		Fresh	Stored
Aldehydes	Traces	Not present	Oxides of nitrogen in conversion to N_2O_5	Traces	Traces
Ammonia and aliphatic amines	0.0002	0.0032	Carbon monoxide	0.029	0.018
Ketones	0.0002	0.001	Sulfur dioxide	0.0004	0.001
Organic acids in conversion to CH_3COOH	0.02	0.018	Hydrocarbons in conversion to C	0.102	0.18
Indole	0.0001	0.00003	Phenols	0.0006	0.0006
Mercaptanes and hydrogen sulfide	0.0005	Traces	Carbon dioxide	1%	1.2%

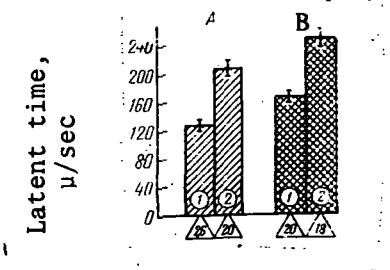

Figure 5. Effect of a Group of Gaseous Toxic Substances, Discharged by Fresh (A) and Stored (B) Feces, on the Latent Time of the Flexor Reflex in White Mice. Designations are the same as in Figure 1.

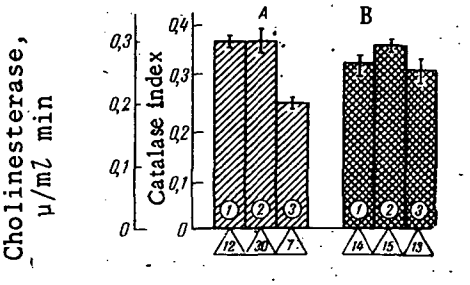

Figure 6. Effect of a Group of Gaseous Toxic Substances, Discharged by Stored Feces, on the Level of Carboxyhemoglobin (A) and the Amount of Acetylcholine (B) in the Blood of White Mice. Designations are the same as in Figure 3.

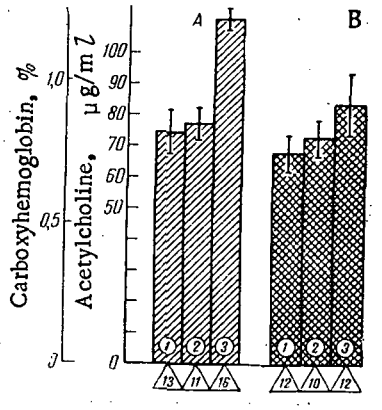

Figure 7. Effect of a Group of Gaseous Toxic Substances, Discharged by Stored Feces, on the Activity of Cholinesterase (A) and Catalase (B) of the Blood of White Mice. Designations are the same as in Figure 3.

The changes which we detected in the acetylcholine-cholinesterase system, just as in the test with the fresh feces, could have led to intensification of the inhibition process in the nervous system, which conditioned the increase in the time of the latent period of the flexor reflex of the experimental mice (Figure 5). /169

Shifts in the acetylcholine--cholinesterase system which we observed during the action of gas discharges from the fresh and stored excrement, indicate certain disorders in the "functional system," which ensures the neuro-humoral equilibrium in the organism (Al'pern, 1963). The physiological importance of these disturbances should be of value in corresponding tests. However, the very fact of the onset of such disorders indicates that the group of gaseous toxic substances which enter the air from the fresh and stored excrements causes not only a stimulating but also a generally toxic action.

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EFFECT OF A CHEMICAL PRESERVATIVE ON THE INTENSITY OF THE DISCHARGE OF CERTAIN GASEOUS TOXIC SUBSTANCES FROM STORED URINE

L. T. Poddubnaya, L. N. Rogatina, V. V. Kustov and V. I. Mikhaylov

ABSTRACT. The effect of a chemical preservative from a class of phenols on the intensity of gas discharges from stored urine was studied.

It was determined that addition of a preservative reduces the entrance of substances of a group of ammonia, ketones, fatty acids and nitrogen oxides into the air. In addition the discharge of carbon monoxide and organic compounds, as determined by the total amount of carbon, did not change.

Earlier we showed that urine which is stored in special containers in /170
small compartments is a potential source of contamination of the air environment by a number of toxic substances which have an unpleasant odor, and a stimulating and generally toxic action (Kustov, Mikhaylov, Poddubnaya, Rogatina, 1967).

In connection with the fact that the majority of gaseous toxic substances formed during microbial decomposition of organic products which are contained in the urine, one way in which the intensity of the gas production can be reduced is the method of treatment of the discharged urine by various antiseptics.

For this purpose chemical compounds from a group of alcohols, the salts of heavy metals, etc. are used. We used a preservative selected by L. N. Rogatina from the phenol class in our tests for studying the problem of the effect of chemical antiseptics on the intensity of production and discharge into the air of toxic substances from stored urine.

Two liters of the collected urine was stored in 20-ℓ hermetically-sealed /171
glass flasks. The preservative is introduced into one of the flasks at a rate of 2.5 g of preparation to one liter of urine.

EFFECT OF THE PRESERVATIVE ON THE INTENSITY OF DISCHARGE OF CERTAIN GASEOUS TOXIC SUBSTANCES FROM URINE WHICH IS STORED FOR TEN DAYS*

	Amount of chemical substances, mg for 100 ml of urine													
	Without preservative							With preservative						
No. of tests	Ammonia and aliphatic amines	Acetone	Organic acids in conversion to acetic acid	Nitrogen oxides in conversion to N_2O_5	Carbon monoxide	Hydrocarbon in conversion to carbon	Phenols	Ammonia and aliphatic amines	Acetone	Organic acid in conversion to acetic acid	Nitrogen oxides in conversion to N_2O_5	Carbon monoxide	Hydrocarbon in conversion to carbon	Phenols
1	—	0,001	0,129	—	—	—	—	0,0025	0,0008	—	0,0034	0,028	—	0,0015
2	0,053	0,007	0,083	0,012	0,012	0,018	0,0010	0,0047	0,0010	0,009	0,0025	0,028	—	0,0011
3	0,016	0,0015	0,490	0,012	0,029	—	0,0035	0,0011	0,0006	0,007	Traces	0,034	0,057	0,0006
4	0,056	0	0,035	0,006	0	—	0,0030	0	0,0013	0,016	.	0,014	0,014	0,0007
5	0,005	0,0026	0,018	—	0	—	0,0012	0,0016	0,0004	0,031	0	—	0,164	0,0007
6	0,125	0,0009	—	0,036	0,012	0,151	0,0005	0	0,0005	0,014	Traces	—	0,204	0,0007
7	0,077	0,0013	0,080	0,004	0,028	0,031	0,0026	0	0,0005	0,007	.	0,024	0,300	0,0007
8	0,093	0	—	Traces	0,059	0,0328	—	0	0,0005	0,016	.	0,014	0,170	0,0006
Average	0,061	0,001	0,139	0,015	0,018	0,173	0,0019	0,0013	0,0007	0,014	.	0,016	0,148	0,0008

Translators' Note: Commas indicate decimal points.

Before introduction of the preservative and near the end of the period of its storage (ten days) we determined the presence of microorganisms, their amount and the species to which they belonged in the urine. On the tenth day of the experiment we withdrew air samples from the flasks for subsequent chemical analysis.

We conducted determination of harmful substances by standard methods, accepted in industrial-hygiene chemistry (Alekseyeva et al., 1954). The amount of toxic substances was calculated for 100 mℓ of urine.

The test which we conducted showed that the amount of microorganisms in the urine increased from tens to billions of microbial bodies per 1 mℓ by the tenth day of storage without addition of the preservative. Introduction of the latter into the urine almost completely retarded the growth of the microorganisms. The preservative effect in the given instance was practically 100%. The amount of microorganisms in 1 mℓ or urine, stored for ten days (mean data from eight tests), is given below:

	Conditions of storage	
	Without preservative	With preservative
Beginning of the test	90.5 ± 15	774.2 ± 85.3
After 10 days	$4.8 \cdot 10^9 \pm 1 \cdot 10^9$	6.25 ± 3.0
Effect of preservative, %	------	99.99

Data on the effect of the preservative and the intensity of production and discharge of certain toxic substances on the urine stored for ten days are given in the table.

From the table it is evident that the addition of the preservative reduced the entrance of ammonia and aliphatic amines, acetone, volatile organic acids, nitrogen oxides, and phenols into the air above the stored urine and did not have any substantial effect on the discharge of carbon monoxide and organic compounds which we determined according to the total amount of carbon.

Our calculations show that, in comparison with the background tests, the preservative under study reduced discharge of substances from the ammonia group

into the air on the average by forty-seven times, for acetone by approximately 1.4 times, for fatty acids by more than nine times. Nitrogen oxides were in the air above the urine, as a rule, in minute quantities, while in the control test their amount in five samplings out of eight fluctuated from 0.004 to 0.036 mg/*l*.

If these data are linked to the bacteriostatic action of the preservative, then one can conclude that the production of the above-mentioned gaseous chemical substances depends basically on the microbial decomposition of urea and other organic compounds which comprise the urine. As far as the carbon monoxide is /173 concerned, its discharge from the stored urine evidently is not connected to the vital activity of the microorganisms, or the introduction of the preservative did not have any substantial effect on its production.

Thus the tests which we conducted show that by using a preservative one can significantly reduce the discharge of certain toxic substances from stored urine and reduce the potential danger of the gas discharges from the urine altogether.

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PRESERVATION OF URINE IN A SYSTEM FOR REGENERATION OF WATER FROM IT

L. N. Rogatina, A. M. Karagodina and V. A. Panchenko

ABSTRACT. Thirty-two substances and their combinations for the preservation of urine during storage for two weeks at 18-20° were studied.

Five prescriptions were studied in the unit for regeneration of water from urine in order to discover possibilities for the use of preservation of urine in the indicated system.

During the preservation of the urine a reduction was noted in the amount of ammonia and the total amount of organic substances in the condensate in comparison with a condensate obtained from the evaporation of unpreserved urine. The condensate corresponded to the GOST [State Standards] requirements for drinking water for the amount of microorganisms, transparency and odor. A small amount of preliminary purification of the condensate on ion exchange resins was required in order to obtain water which corresponds to the GOST requirements for drinking water based on the physical-chemical indices.

At the present time different life-support systems based on the complete or /173 partial regeneration of basic substances are being developed for continuous spaceflight conditions: oxygen, water, food products, i.e., the conversion of certain end products into a form suitable for further use. A promising system for such flights is one which would include a cycle for regeneration of water, because during prolonged flight the amount of water which is necessary daily for human beings is 2.5 to 3.0 l. One of the starting products for the production of drinking water could be urine.

The basic components of urine are: urea, sodium chloride, ammonia, etc. All other substances (phosphates, sulfates, creatinine, creatin, etc), are present in insignificant amounts; however, a complete list of the substances which make up urine has not yet been fully clarified.

According to the data of many authors, freshly eliminated urine of healthy adults contains microorganisms. The number of the microorganisms per ml

CHARACTERISTICS OF THE URINE AND CONDENSATE OBTAINED FROM THE URINE WITH THE USE OF VARIOUS PRESERVATIVES.

Indices*		Copper sulfate in g/l and perhydrol, 2 g/l	Chromium anhydride in sulfuric acid, 2.49 ml/l	Sulfuric acid, 2 ml/l and perhydrol, 2 g/l	Paranitrophenol in sulfuric acid, 0.1 g/l	Formalin 1.5 ml/l, caustic soda 0.5 g/l	Without preservatives
Amount, l	Urine	37.0	30.0	35.0	15.0	12.0	10.0
	Condensate	30.0	24.0	30.0	12.0	9.0	7.5
Odor, grades	Urine	Aromatic, 3	Aromatic, 3	Aromatic, 2	Aromatic, 3	Aromatic, 3	Specific, 5
	Condensate	0	0	0	0	0	2
Color	Urine	Dark green	Dark green	Transparent	Transparent	Transparent	Weak suspension, of a yellow color
	Condensate	0	0	0	0	0	Murky
Amt. of microbial cells in 1 ml	Urine	0	0	0	0	0	1,300-27
	Condensate	0	0	0	0	0	$2 \cdot 10^5$
pH	Urine	4.7	4.0	2.5	5.0	11.0	6.0
	Condensate	4.0	5.1	5.2	5.6	8.0	7.0
Permanganate oxidizability, in mg/l O_2	Condensate	3.1	6.1	7.9	29.1	28.7	50.0
Ammonia, mg/l	Condensate	13.5	6.6	5.9	23.1	21.8	100.0

*The data of chemical and bacteriological investigation of the urine and condensates characterize the condition of the objects after exposure time of two to three hours both in the test and in the control.

fluctuates and depends on individual peculiarities of the person. Thus, according to the data of Philpot (1956) and others, the urine of adults was not found to be sterile in 82% of the men and in a study of the urine of women /174
in 34% of the cases.

During the storage of urine, protein components and certain other component parts of it decomposed as a result of the vital activity of the microorganisms, and as a result different harmful toxic substances may enter the air: hydrogen sulfide, indole, ammonia, etc. In using a system for the regeneration of water from urine we have not excluded the possibility of storage of the urine before it enters the regeneration unit. In this period the urine may undergo decomposition. Consequently, in order to obtain water suitable, in a bacteriological sense, for drinking purposes, the multiplication of microorganisms in it must be halted. This can be achieved by preliminary chemical treatment of the urine, i.e., by its preservation. The principal aim of preservation is: reduction of the total microbial contamination and cessation /175
of the vital activity of the microorganisms in the urine during its storage, and decontamination in the case of casual entrance of pathogenic flora;

removal of the mechanical contaminants in the urine (mucus, protein);

binding of certain components of the urine into strong chemical compounds (urea, ammonia, etc);

maintenance of a definite pH value for the preserved urine.

Various chemical substances and their combinations may be used for preservation of urine. The best combination depends on a great number of factors. In selection of the preservant for a system of regeneration of water from urine it is necessary to consider that:

the substance must have a sufficiently high effectiveness in relation to /176
the vegetative microorganisms (*Escherichia coli* and Staphylococcus aureus), inhabitants of the urinary tracts of man, and it must also inhibit the vital activity of microflora during storage of the urine for two weeks at a temperature of 20 ± 5°;

the substance must not have an odor and must not form additional odors during storage with components of urine;

the substance must not lose its antibacterial properties during contact with materials used in the system for regeneration of water from urine and must not cause changes of the physical-properties of materials of the system;

at the same time the amount of the preservative must be minimal.

We studied thirty-two substances from various classes of chemical compounds in order to discover the possibilities for their use in preservation of urine. Many of them did provide a preserving effect for a prolonged amount of time: up to a year and longer. However, not all of the substances which we studied could be used for chemical processing of the urine in the system for regeneration of water from it.

For this purpose we previously selected five prescriptions: copper sulfate and perhydrol, a mixture of chromium anhydride and concentrated sulfuric acid, concentrated sulfuric acid and perhydrol, paranitrophenol, formalin and caustic soda.

Study of the preserving action of the chemical reagents was conducted in a unit intended for investigating the process of removal of moisture from the surface of the materials in an air current in a closed circulating system with subsequent condensation.

Before feeding the urine into the regeneration unit it was subjected to preservation by one of the selected prescriptions, and then we allowed the preserved urine to pass through the device for six to seven hours.

The effectiveness of the preservatives used in the system for regeneration of water from urine was evaluated according to the following indices:

the antimicrobial action of the urine during storage and processing of the urine in the regeneration system;

the degree of binding of certain components of the urine (urea, ammonia);

the total amount of organic substances in the condensate obtained during the evaporation of the urine in the water regeneration unit;

the pH value of the condensate;

intensity of the odor of the condensate.

We determined the number of microorganisms in 1 ml, the color, odor, and acidity in the starting urine which was preserved and later regenerated. In the resultant condensate, we determined the oxidizability and the amount of ammonia in addition to these studies. /177

Results of our investigations are presented in the table. As can be seen from the data presented in the table, when we used copper sulfate with perhydrol, mixtures of chromium anhydride with sulfuric acid, concentrated sulfuric acid with perhydrol, perinitrophenol, and formalin with caustic soda as preservants for the urine in the system for regeneration of water from urine we observed a reduction in the amount of ammonia, the total amount of organic substances, a disappearance of the odor and murkiness in the condensate obtained in comparison with the condensate that resulted after evaporation of the unpreserved urine.

All of the enumerated preservants in the test dosages had a bactericidal action on microorganisms in the urine, and the microorganisms did not separate out during contamination on nutrient media.

The best results were obtained by the use of sulfuric acid with perhydrol and also by a solution of chromium anhydride in sulfuric acid as a urine preservative. By use of the indicated preservant the oxidizability of the obtained condensate reduced to 6.1-7.9 mg/l of O_2, the amount of ammonia reduced to 5.9-6.6 mg/l, in comparison with the condensate obtained by distillation of unpreserved urine, and the pH of the solution was maintained at an interval of 5.0-5.5 (average was 5.2). The optimum amounts of the preservant of sulfuric acid (2 ml/l) and perhydrol (2 ml/l), and also of chromium anhydride in sulfuric acid (2.49 ml/l), which we used for preservation of the urine, are completely acceptable for regeneration systems in space aircraft.

A condensate produced in the evaporation unit by using copper sulfate and perhydrol, sulfuric acid and perhydrol, chromium anhydride in sulfuric acid, paranitrophenol, formalin and caustic soda as a preservant, corresponding to

the GOST requirements for drinking water based on the amount of microorganisms in it, transparency and odor. However, it did not meet the GOST requirements for drinking water based on the amount of ammonia; therefore some preliminary purification of it on ion exchange resins is necessary.

THE RESISTANCE OF ANIMALS TO TOXIC ACTION OF CERTAIN GASES AFTER ADAPTATION TO HYPOXIA

G. A. Vasil'yev, L. A. Tiunov and V. V. Kustov

ABSTRACT. In tests of white rats the resistance of animals which had been adapted to hypoxia to the toxic action of carbon monoxide, nitrogen oxide, triethylamine and freon-12 were studied. It was found out that preliminary adaptation of the animals to hypoxia increases their resistance to acute poisoning by carbon monoxide, nitrogen oxides and freon-12 and does not change the sensitivity of the mice to the toxic action of triethylamine. It was assumed that adaptation of the animals to hypoxia may substantially increase the resistance to the toxic action of posions, which disturbs the oxygen balance in an organism by one means or another.

There is a significant number of investigations in which the preventive /178
effectiveness of adaptation to hypoxia is shown in relation to diverse harmful influences. Thus, rats which have been conditioned to hypoxia are significantly better able to withstand cooling and overheating (Agarkov, 1962; Terapata, 1962), and in cats resistance to severe thermal shock is increased (Gubler, Fenster, 1960). Adaptation to hypoxia increases the resistance of experimental animals to the action of radial accelerations (Sirotinin, 1962, 1964; Petrukhin, 1968; Uglova, 1968), to hypocapnia (Kochetov, 1968), to acute hypoxia and vestibular irritations (Malkin, Yukhnovskiy, Markaryan, 1968), to ionizing radiations (Barabasheva, 1955, 1955a, 1956; Vasil'yev, 1958, 1958a, 1960; Belokonskiy, 1959; Paribok et al., 1961; Rambach and others, 1954; Konecci et al., 1956). At the same time information on the change in resistance to the toxic action of varying harmful chemical substances due to the influence of adaptation to hypoxia has always been limited (Tiunov, Vasil'yev et al., 1960, 1962). The aim of the present study consisted in experimental investigation of the effect of adaptation to hypoxia on the resistance of animals to carbon monoxide, nitrogen oxide, freon-12 and triethylamine (TEA). These substances can enter the atmosphere of hermetically sealed cabins of aircraft in the course of human respiration (carbon monoxide, triethylamine), or may be

given off by technical systems (freon-12), or may enter the air during thermal destruction of certain nonmetallic materials (Klemedson, 1962).

All of the enumerated toxic substances have a different and comparatively thoroughly studied mechanism of action on an organism. Therefore their use in our experiments would promote clarification of certain aspects of the mechanism of the increase of resistance of an organism by means of adaptation to hypoxia. Our study was conducted on white male mice weighing 19-21 g. The animals were subjected to a two-hour inhalation inoculation by toxic substances using the dynamic method. The experimental mice had previously adjusted to hypoxia in a thirty-day period according to the following plan: in each conditioning test in the pressure chamber the "elevation" was constant, but it gradually increased, reaching the maximum by the middle of the thirty-day training period. The /179
initial "elevation" of the ascent was 5,000 m, and the maximum was 7,000 m. The duration of each training test was two hours. Inoculation was carried out twenty-four hours after the last test in the pressure chamber.

The investigation could be limited to inoculation of the animals with any one concentration of poison. However, we thought it best to study the action of several concentrations for each poison, because this enabled us to conduct a more detailed quantitative analysis of the mechanism of the protective action of adaptation to hypoxia.

The acute inhalation inoculations of the mice were made in a glass desiccator with a capacity of 8 l. The air containing the toxic substances was drawn through the desiccator by the aid of twin alternately working air blowers at a rate of 1 l/min.

We added the carbon monoxide to the inoculation chamber from a Douglas bag in the form of an earlier-prepared mixture containing air; freon-12 in the form of a mixture with air and oxygen (amount of oxygen in the mixture reached up to 21% in all cases).

The nitrogen oxides entered the desiccator directly from a gas tank by the aid of a specially designed automatic metering apparatus (Vasil'yev, 1962).

EFFECT OF ADAPTATION TO HYPOXIA ON THE RESISTANCE OF WHITE MICE TO INHALATION ACTION OF CARBON MONOXIDE, NITROGEN OXIDE, TRIETHYLAMINE AND FREON-12*

Animals	Concentration of CO, mg/l	Concentration of nitrogen oxide in conversion to N_2O_5, mg/l	Concentration of TEA, mg/l	Concentration of F-12, % by vol.	No. of mice			Percent of destruction	Samples
					Total	Destroyed	Survived		
Experimental	6,0	—	—	—	10	10	0	100	6,96
	4,0	—	—	—	10	3	7	30	4,48
	3,0	—	—	—	10	2	8	20	4,16
	2,5	—	—	—	24	3	21	12	3,87
Control	6,0	—	—	—	10	10	0	100	6,96
	4,0	—	—	—	10	6	4	60	5,25
	3,0	—	—	—	10	4	6	40	4,75
	2,5	—	—	—	24	10	14	40	4,75
Experimental	—	1,5	—	—	17	14	3	82	5,92
	—	1,1	—	—	17	7	10	41	4,77
	—	0,9	—	—	17	2	15	13	3,87
Control	—	1,5	—	—	17	17	0	100	6,96
	—	1,1	—	—	17	12	5	71	5,55
	—	0,9	—	—	17	6	11	35	4,62
Experimental	—	—	3,6	—	20	15	5	75	5,67
	—	—	2,4	—	20	5	15	35	4,67
	—	—	2,0	—	20	6	14	30	4,48
	—	—	1,0	—	20	1	19	5	3,36
Control	—	—	3,6	—	10	7	3	70	5,52
	—	—	2,4	—	10	4	6	40	4,75
	—	—	2,0	—	10	2	8	20	4,16
	—	—	1,0	—	10	0	10	0	3,04
Experimental	—	—	—	78	5	3	2	60	5,25
	—	—	—	70	15	2	13	13	3,87
	—	—	—	63	5	0	5	0	3,36
Control	—	—	—	78	5	5	0	100	6,64
	—	—	—	70	15	10	5	66	5,41
	—	—	—	63	5	2	3	40	4,75

*Translators' Note: Commas indicate decimal points.

Triethylamine vapors were supplied to the chamber from an "ogee," in order that the stream of air would flow through it at a rate necessary for creation of a given concentration in the desiccator.

During the inoculations in the desiccator the air temperature was maintained at a level of +20 to +22°, the amount of oxygen at 19-20%, and the amount of CO_2 at 0.3 to 0.5%.

The air samples for chemical analysis were taken directly from the desiccator. The amount of carbon monoxide was determined by a LIOT titration device (Kozlyayeva, 1949), and the amount of nitrogen oxide was determined by the colorimetric method on the FEK-56 with the aid of a Grissa-Ilosvaya reagent. To determine the amount of triethylamine vapor in the air we used the method suggested by G. S. Salyamon (1962) for quantitative analysis of alkylamine. Freon-12 was determined by the method devised by O. D. Kahlizovoy and Ye. I. Vorontsova (1952).

The death of the mice during the inoculation process and for five days following its completion were recorded. Five experimental and five control animals were taken simultaneously for each test. Data on the effect of adaptation to hypoxia on resistance to the action of carbon monoxide, nitrogen oxide, triethylamine and freon-12 are presented in the table. Results of the acute inoculations were subjected to processing by the Miller and Taynter sample analysis method (Belen'kiy, 1959). The working samples, which corresponded to percentages of destruction, were plotted on a logarithmic smapling grid; later, along a straight line made by these points, we computed the CL_{50} and its constituent boundaries for the experimental and control animals. /181

It turned out that the mice which had adapted to hypoxia were significantly more resistant than control animals to the toxic action of carbon monoxide, nitrogen oxides and freon-12. Thus, the CL_{50} for the control group of mice which had been inoculated with carbon monoxide was 3.2(2.64-3.76) mg/ℓ, and for the animals which had adapted to hypoxia it was 4.5 (3.92-5.08) mg/ℓ, at $r = 0.05$.

The CL_{50} was 0.9 (0.85-1.03) mg/ℓ for the control animals during inoculation by nitrogen oxides, and for the experimental animals it was 1.15

(1.06-1.24), at r = 0.05. The CL_{50} for the experimental mice during inoculation by freon-12 was 76 % by volume (72.85-79.15), and for the control mice it was 66% by volume (61.38-70.62), at r = 0.05. During inoculation by the triethylamine we detected no protective effect of the adaptation to hypoxia.

The increase in the resistance of the experimental animals to acute poisoning by carbon monoxide and nitrogen oxide is in conformity with the concepts of the large role of specific reactions to hypoxia in the mechanism of the protective effect of adaptation to hypoxia. Carbon monoxide and nitrogen oxides are, as is known, hypoxic poisons. The fact of the absence of the protective effect of adaptation during poisoning by the triethylamine does not contradict these concepts. On the contrary, the selective nature of the protection, evidently related to differences in the mechanism of the action of the poisons, supports these concepts. The mechanism of the toxic action of triethylamine should not be considered as finally established. At the present time there is information available which indicates the lack of a direct connection of this mechanism to the development of hypoxia.

The increase in the resistance of the animals which have been adapted to hypoxia to the toxic action of freon-12 is also clarified by the mechanism of the action of this poison. Freon-12 has a narcotic action, and acute intoxication by this poison, as by any narcotic, is accompanied by a change in the oxygen makeup of an organism. The data which we obtained confirm the fact that adapation of white mice to hypoxia substantially increases their resistance to the toxic action of poisons and by this or some other means conserves the oxygen balance in the organism.

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THE TOXIC ACTION OF GASEOUS PRODUCTS OF THE VITAL ACTIVITY OF AN ORGANISM

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ABSTRACT: The peculiarities of the biological action of a group of gaseous products of the vital activity of white rats in twenty-six-day experiments were studied. It was established that this group fo chemical substances causes damage to the pulmonary tissue and development of anemia in the experimental animals, and leads to an increase in oxygen consumption, increase in weight of the thyroid gland, inhibition of the growth of the experimental rats, and changes the catalase activity of the blood. Using the data in the literature and the material from their own study the authors notw a number of characteristic peculiarities in the biological action of a group of gaseous products of the vital activity of an organism.

Investigation of the toxic action of a group of gaseous products of /182
vital activity involved serious methodological difficulties. At the present time more than 400 chemical substances have been identified and in a number of cases have been quantitatively determined to be in the composition of the so--called "metabolic eliminations;" 149 compounds have been discovered in discharges from the oral cavity, 271 enter the atmosphere from skin contamination, 183 substances are eliminated with the urine and 196 with the feces (Beber, /183
1967).

It is very difficult to officially create such a set of chemical substances for investigation of their toxicity for varying time periods of influence on an organism. Therefore we considered it best to conduct a study of peculiarities of the toxic effect of products of the vital activity under experimental conditions such that the entire set of the substances sould be generated in the atmosphere of a chamber by the experimental animals themselves.

Such conditions could be created by placing the animals in a hermetically sealed chamber which would not be cleaned throughout the entire period of the experiment. Gaseous chemical substances would accumulate in the atmosphere

of the chamber both through elimination from the animals themselves and by entering the air from their feces and urine. A part of these compounds would be removed with the condensate before establishment of a definite equilibrium. However, we created the maximally severe conditions in the chamber in order to study peculiarities of the toxic action of the entire group of substances which are found during the process of the vital activities of an organism.

We tested white rats of both sexes weighing 100-150 g. The inoculation was carried out in a metallic hermetic chamber with a volume of 0.24 m^3. Twenty rats were used at one time in the test. The amount of gaseous products of vital activity in the chamber was monitored on the basis of the most typical metabolic poisons. We determined the amount of ammonia with Nesler reagent (Zhitkova, 1954), the amount of alkylamines by the Salyamon method (1965), the amount of acetone by the F. D. Shakhvarger method (1954), the amount of phenols colorimetrically with diazotized paranitroaniline (Yefremova, 1954), the amount of indole by the colorimetric method with sodium nitrite and nitric acid. In addition, we determined the amount of carbon dioxide with the aid of a UG-2 (Filyanskaya, 1960) and the total amount of organic oxidizable substances by the bichromate method, which has the fewest shortcomings in comparison with the permanganate and iodate methods (Ballod, Yegorova, Nefedov et al., 1967). The amount of oxygen and carbon dioxide in the chamber was controlled by the aid of an Ors-Lunge device.

The duration of the test was twenty-six days. The excess carbon dioxide was removed from the chamber by means of a KhPI absorbant, and the amount of CO_2 in the air of the chamber did not exceed 1% during the experiment. Oxygen was supplied to the chamber automatically. Its concentration was maintained at a level of 19%.

The control group of rats was kept in a vivarium. The study of the vivarium atmosphere showed the presence of the same products of metabolism in it as in the inoculation chamber, but their amount was constant and not very great. Thus, /184 for example, out of seven air samples taken at different times in the vivarium, ammonia was not detected at all in three of these samples, and in four of them its content fluctuated from 8 to 16 mg/m^3.

Data on the amount of gaseous products of vital activity in the inoculation chamber during the tests are presented in Table 1.

TABLE 1. AMOUNT OF GASEOUS PRODUCTS OF METABOLISM IN THE INOCULATION CHAMBER.

Substance	Concentration, mg/m^3		Substance	Concentration, mg/m^3	
	Max.	Min.		Max.	Min.
Carbon monoxide	50.0	14.0	Acetone	2.7	0.3
Ammonia	119.2	22.3	Phenol	0.26	0.05

The animals were weighed periodically (once a week). Dynamics of the changes in their weight are presented in Figure 1.

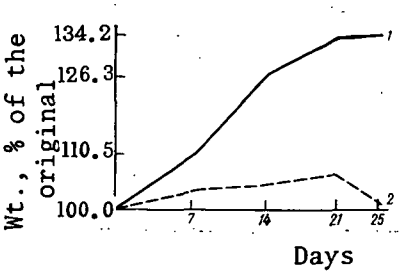

Figure 1. Change in Weight of the White Rats Under the Influence of Gaseous Products of Vital Activity. 1, Control; 2, Test.

As can be seen from Figure 1, appearance of the toxic action of the group of gaseous products of the vital activity could be noticed beginning from the first week of hermetization, which was expressed in inhibition of the growth of the animals.

The test was stopped on the twenty-sixth day of hermetization. We studied the intensity of the oxygen consumption by the S. V. Miropol'skiy method in ten of the experimental animals and ten control animals. The results obtained are presented in Table 2.

From the data in Table 2 it is evident that a statistically reliable increase in oxygen consumption can be noticed by the twenty-sixth day of the experiment for the experimental animals.

Then the experimental and control animals were decapitated and subjected to pathomorphological, biochemical and hematological examinations.

TABLE 2. EFFECT OF GASEOUS PRODUCTS OF THE VITAL ACTIVITY ON INTENSITY OF OXYGEN CONSUMPTION BY WHITE RATS.

Test		Control	
Number of the animal	Consumption of O_2 for 100 g of wt	Number of the animal	Consumption of O_2 for 100 g of wt
1	13.28	11	4.48
2	11.00	12	4.56
3	9.48	13	5.75
4	7.03	14	4.83
5	11.55	15	6.37
6	8.96	16	5.09
7	6.75	17	5.45
8	9.62	18	4.47
9	9.10	19	4.80
10	11.4	20	5.59
M	9.82 ± 0.21		5.14 ± 0.19
P		< 0.001	

The numerical data were subjected to statistical analysis by the Student and Fisher method.

Data of the hematological investigations are presented in Table 3. From Table 3 it can be seen that a statistically /185 significant reduction in the amount of erythrocytes in the peripheral blood can be noticed in the experimental animals. These data are in conformity with observations of Tomas and Bek (1967), who detected the phenomenon of hemolysis and the appearance of Heinz bodies in the blood during the combined influence of metabolic poisons (indole, skatole, hydrogen sulfide, methylmercaptan).

We made a determination of the SH-groups in the homogenates of the spleen and in the blood by amperiometric titration using the Kolthoff and Harris method (1946) during a biochemical examination of the experimental and control animals; we also studied the regenerated glutathione in the blood by the Benesch method (1950); we studied the catalase activity of the blood by the A. N. Bakh and R. S Zubkova method.

We detected a small increase in the amount of regenerated glutathione in the blood. The amount of sulfhydrol groups in the spleen and in the blood of the experimental animals did not differ practically from the control group. We noticed an increase in the catalase activity of the blood in the experimental rats. The catalase index of the blood was eleven on the average, and in the control animals it was 0.74.

TABLE 3. EFFECT OF A GROUP OF GASEOUS PRODUCTS OF THE VITAL ACTIVITY ON THE AMOUNT OF ELEMENTS FORMED IN THE BLOOD OF WHITE RATS.*

Test								Control							
Erythrocytes	Leucocytes	Lymphocytes	Segmental-nuclear	Bacillus nuclear	Eosinophils	Basophils	Monocytes	Erythrocytes	Leucocytes	Lymphocytes	Segmental-nuclear	Bacillus-nuclear	Eosinophils	Basophils	Monocytes
4,2	2630	62	35	1	1	—	1	5,6	5350	83	15	1	—	—	1
3,8	2400	86	12	1	—	—	1	4,1	4250	83	15	1	—	—	1
3,6	5650	69	26	2	—	1	2	5,4	3500	80	16	2	1	—	1
4,9	2900	71	27	1	—	—	1	5,6	4500	77	20	2	—	—	1
4,1	2400	74	23	2	1	—	—	5,7	4250	76	20	1	1	—	2
2,4	7450	70	27	—	1	1	1	3,5	5050	82	14	2	—	—	2
4,9	6200	73	24	—	1	1	1	5,9	6100	80	18	—	1	—	1
3,8	3500	73	24	1	—	—	2	6,7	5750	79	16	1	1	1	2
5,5	6000	—	—	—	—	—	—	7,2	8750	—	—	—	—	—	—
3,2	7020	—	—	—	—	—	—	6,1	3800	—	—	—	—	—	—
4,04	4617	72,25	24,75	1,0	0,5	0,375	1,125	5,58	5130	80,00	16,75	1,25	0,50	0,13	1,37
M ±0,31	±1639	±3,05	±2,8					±0,38	±1380	±0,8	±0,7				

*Translator's note: Commas indicate decimal points.

Determination of the indices for the internal organs of the experimental animals were made before the morphological investigation. Their values are given in Table 4.

TABLE 4. WEIGHT INDICES FOR THE INTERNAL ORGANS OF WHITE RATS, WHICH HAVE BEEN SUBJECTED TO INOCULATION BY GASEOUS PRODUCTS OF VITAL ACTIVITY.

Organs	Test	Intact animals (Rylova, 1964)	Organs	Test	Intact animals, (Rylova, 1964)
Lungs	7,91±0,44	2.8—6.75	Thyroid gland	0.97±0,09	0.15—0.55
Heart	4.94±0.14	2.85—4.4			
Liver	38.37±1,77	28.2—43,5	Adrenal glands	0.35±0,03	0,24—0,38
Kidneys	9.44±0.28	6.3—8.36			

As can be seen from the data in Table 4 the weight indices of a number of the organs of the experimental rats were increased. There was an especially noticeable increase in the weight index of the thyroid gland. The increase /187 in the weight indices of the lungs and heart are also of interest.

We detected phenomena of acute emphysema, the lack of typical alveolar complexes, expansion of the alveolar cells, rupture of individual membranes between them with the formation of spore-like projections (Figure 2) during /188 histopathological investigations in the lungs of the experimental animals. In the kidneys we found a large amount of protein fluid in the lumina of the convoluted tubules; there was sudden hemorrhaging of the kidney vessels up to the glomerular capillaries (Figure 3).

In a special experiment we traced the change of certain of the indices studied during a more pronounced action of the group of gaseous products of vital activity. It turned out that the reduced amount of erythrocytes, which we detected in the twenty-six day experiment, alternated with erythrocytosis. The number of erythrocytes in one m^3 of blood reached 7.1 ± 0.20 millions, and the catalase index of the blood decreased to 0.59 ± 0.004; the intensity of consumption of oxygen by the experimental animals remained high (7.2 ± 1.2 mℓ O_2 for 100 g of weight).

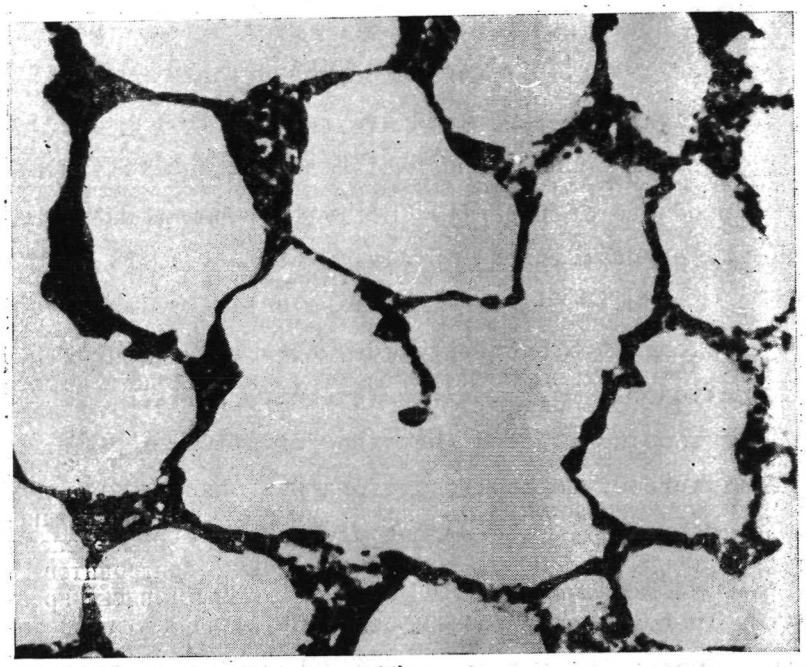

Figure 2. Microphotograph of the Lungs. Vesicular emphysema. Elongated alveoli and membrane remnants are evident. The stain is hematoxylin-eosin. 200 ×

Comparison of the results of the experiments conducted with the data in the literature enabled us to project, in our opinion, a number of characteristic peculiarities in the biological action of the entire group of gaseous products of the vital activity of an organism. This set of foul-smelling, irritating and anoxic poisons causes damage to the pulmonary tissue and development of anemia in the experimental animals during prolonged continuous action and leads to an increase in the consumption of oxygen. The increase in the weight of the thyroid gland gives confirmation of the development of its hyperfunction. This is in accordance with data on the increase of the gas exchange and inhibition of the growth of experimental animals. /189

Articles in the literature also indicate that the experimentally induced increase of the thyroid gland in rabbits is accompanied by inhibition of their growth, by increased frequency of pulmonary respiration, and by an increase in

consumption of oxygen (Kakhana, 1968). Changes in the catalase activity of the blood should be noted among other signs of the toxic action of the gaseous products of vital activity. It can be supposed that these cyclical changes in the catalase activity of the blood are related to the development of hemolysis. At least three compounds which suppress catalase activity are found in the group of products of the vital activity. Among them are: hydrogen sulfide (Nedolis, 1960), acetone (Dienes, Tofalvis, Csotos, 1965), ammonia (Kustov, Mikhaylov, 1966). Reduction in the catalase activity leads to the appearance of effects of endogenic hydrogen peroxide which induces decomposition of hemoglobin, the formation of Heinz bodies (Brenner, Allison, 1953) and later leads to hemolysis. It should be noted that the formation of Heinz bodies under the influence of a mixture of metabolic poisons was observed by A. Tomas and K. Bek (1967). The decrease in the amount of erythrocytes leads evidently to development of compensatory reactions, such as increase in consumption of oxygen, adaptive increase in catalase activity of the blood, and development of erythrocytosis. Continuing action causes exhaustion of the compensatory mechanisms. These data may be supplemented by information in the literature on the disturbance to the higher nervous activity during the action of gaseous metabolic discharges (Naumova, 1960), on the effect of certain ones on the the acetylcholine metabolism (Kustov et al., this collection), and on the development of stress reactions during intoxication of the animals by a mixture of certain products of vital activity (Tomas, Bek, 1967).

In conclusion, it should be noted that study of the biological action of a group of gaseous products of the vital activity of an organism requires further investigation.

In the present article we have only attempted a generalization of our own information and data in the literature on this problem.

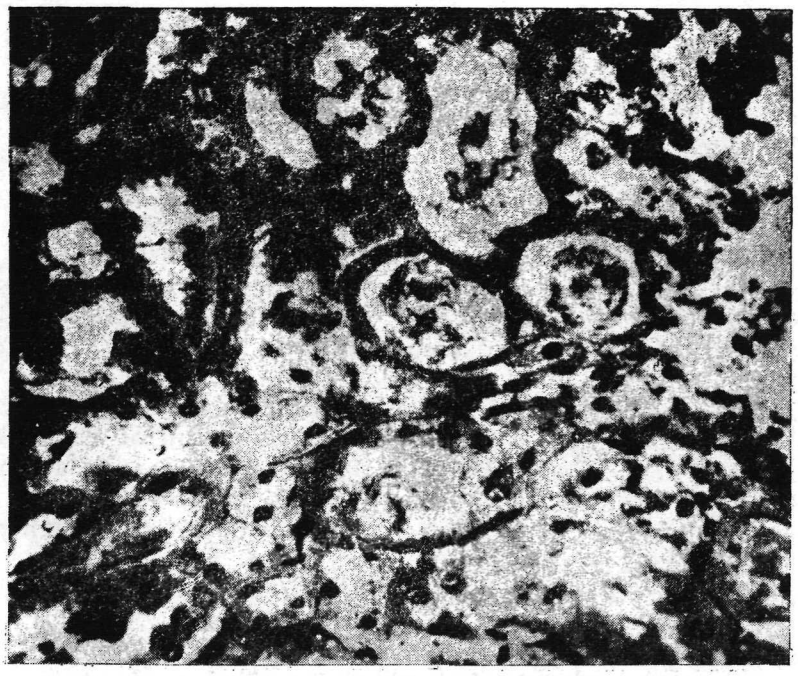

Figure 3. Microphotograph of the Kidneys. Protein fluid in lumina of the convoluted tubules. Part of the tubules are elongated and their epithelium is compressed. Stain is hematoxylin-eosin. 400 ×

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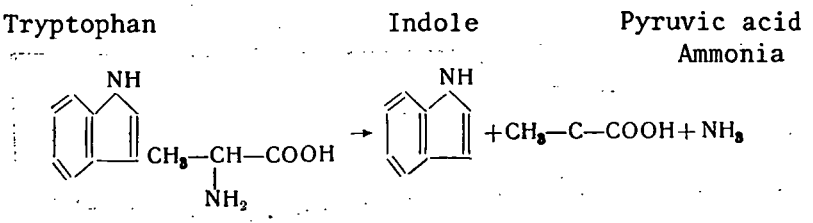
PROBLEM OF STUDYING THE TOXICITY OF INDOLE

A. K. Sgibnev and T. A. Orlova

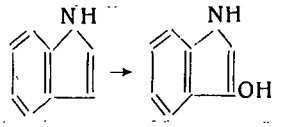
ABSTRACT: Inhalation of indole vapors in a concentration of 9.0 to 10.0 mg/m^3 for two to three hours causes no substantial changes in the organism of mice, rats and rabbits. After intravenous injection of rabbits with a 10.0 mg of an alkaline solution of indole the latter is quickly rendered harmless and expelled from the organism. The threshold for perception of the unpleasant odor of indole is 0.45 mg/m^3. Inhaling the indole vapors in the concentration of more than 1.0 mg/m^3 may lead to negative subjective sensations: headache, nausea.

At the present time investigations which study the toxicity of substances /190
which make up the gas composition of an air medium of hermetic enclosures have great importance. One of the principal sources of harmful impurities in the air under conditions of hermetization is the human metabolic discharges. It is known that from the feces of man alone 196 chemical compounds are given off, including indole (Veber, 1967). The probability of intestinal gases entering the atmosphere of a hermetically sealed enclosure sharply increases in the case of defects of the sanitation device.

I. I. Mechnikov in 1903 was the first to refer to the appearance and fate /191
of indole in an organism. Indole is formed from tryptophan when acted upon by intestinal bacteria. By means of the tryptophanase reaction tryptophan converts to indole with the discharge of pyruvic acid and ammonia (Mayster, 1961):



The greater part of the indole, having been absorbed into the blood from the intestine, enters the liver along the portal vein, where it is rendered harmless, oxidized to indoxyl, and expelled along with the urine in the form of indican.



Indole Indoxyl

The daily urine contains from 0.1-38.0 to 40.0-150.0 mg of indican (Gorzheyshi, 1967). The level of indican in the blood is from 0.03 to 0.16 mg percent (Todorov, 1960). A smaller part of the indole is removed with the feces. According to the data of Ury (1904) and Combe (1907) 7-25 mg of indole are removed daily in the feces, and according to the data of Klemedson (1959) 60-100 mg are removed. 0.013 mg of indole is discharged into the air daily from 100 g of fresh feces (Kustov, Mikhaylov, Poddubnaya, see this collection).

There is information on the effect of indole on the organism of man and animals during parenteral administration and through the gastrointestinal tract in Soviet and foreign literature. Herter (1898), following intravenous injection of dogs with 50-100 ml of 0.1% solution of indole, observed suppression of heart activity, general prostration, narrowing of the pupils, and clonic cramps. After feeding the dogs with indole in a dose of more than 2 g many authors (Baumann, 1886; Jaffe, 1877; Nensky, Siber, Schumov-Simanowsky, 1898) noticed a number of disease phenomena in the animals including hematuria. In 1932 Byungler suggested that indole may play a definite role in pathogenesis of malignant growth. He established that prolonged injection of indole into mice (for six months) causes increase of the hemoporetic tissue in them with an aleukemic blood pattern. The effect of indole on the nervous system was studied by S. D. Bladychko (1911). According to his observations injection of two drops of a 2% solution of indole in olive oil for sixty-three days into the stomach of rabbits caused destructive changes in brain vessels. /192
The degree of change in the nerve elements corresponded to the affection of the vessels which supplied a given section of the brain. According to the

material of Herter (1898), the intake of 2 g of indole by man caused headaches and disturbances to sleep. Repeated intake of 1.5 g of indole for four days. did not cause disease phenomena.

The data of Tomas and Bek (1967) are further confirmation that inhalation of substances found in the composition of the intestinal gases may cause poisoning of an organism. A ninety-day continuous action of a mixture of indole, hydrogen sulfide, mercaptan and skatole in concentrations equal to an eight-hour PDK on animals (monkeys, mice and rats) led to the death of 99% of the animals. The presence of indole vapors in inspired air has an oppressive effect on the emotional condition of man (Bronshteyn, 1950). The extremely unpleasant odor of indole may cause nausea in persons and other negative subjective sensations (Lazarev, 1954). We did not find any other information in the literature on the effect of indole on the organisms of animal and man after its entrance through the upper respiratory tract and on the threshold for perception of odors. The present study is devoted to the study of these problems.

We used several methodological procedures in order to create the given concentration of the substance. The air was sucked through a column of crystalline indole, through liquid indole and finally through a saturated solution of indole in 50% ethyl alcohol. We managed to create the greatest concentration (0.009-0.01 mg/ℓ by the constant blowing of air through the indole (around 1 g), which was placed on a metallic grid inside a chamber. Increase in the amount of the substance and the length of time air was drawn through it did not affect the concentration of indole vapors in the air. Evidently, for the given concentration the air space in the chamber was completely saturated by the indole vapors.

The concentration of indole in the air was determined by the L. A. Mokhov and N. S. Mareyeva method (1962). For this purpose, one liter of air was sucked through 5 mℓ of 50% ethyl alcohol at a rate of 0.15 ℓ/min. We added 0.04 mℓ of concentrated acetic acid and just as much sodium acetate to the solution

obtained. After five minutes we made a photocalorimetric measurement and a comparison with a standard scale.

We set up two series of tests on twenty mice and fifteen rats in order to study the possibility of acute inhalational poisoning by indole vapor. We inoculated the animals in a hermetic chamber with a volume of 100 l by the static method with an exposure time of two hours. The concentration of indole vapors in the chamber was 0.009-0.01 mg/l. In the first ten-fifteen minutes after beginning of the inoculation both the mice and the rats showed some unrest. Later the behavior and condition of the experimental animals did not differ from the control animals. We did not observe destruction of the mice /193
and rats throughout the fourteen days following inoculation.

On the basis of these experiments we figured that indole did not show a pronounced toxic action during acute inhalation poisoning in the test concentrations. Evidently the organism has sufficiently vigorous systems for rendering it harmless.

In order to better understand the supposition that we expressed, we set up test series III on five rabbits weighing 3.5-4.0 kg. The rabbits were subjected to inoculation with an exposure time of three hours at the same concentration of indole with the difference that the substance in the respiration zone was supplied through a special mask. Immediately following the inoculation we determined the amount of indole in the blood plasma of the rabbits according to the Snell method (Snell and Snell, 1937). Blood was taken from an outer vein of the ear. We constructed spectrophotometric curves of the blood plasma with known concentrations of indole in order to determine the amount of indole in the blood: 0.31, 1.25, 6.25 mg %. Analysis of the spectrophotometric characteristics of the plasma showed that the presence of a "peak" in the range of 570 mμ (Figure) is characteristic for indole. The spectrophotometric characteristics of the blood of the inoculated animals did not differ from the intact animals.

In this way, results of this series of tests showed that inhalation of indole in concentrations of 0.009 mg/l did not lead to its accumulation in the blood of the rabbits.

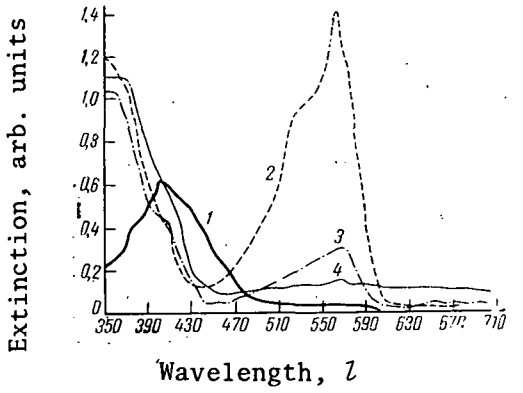


Figure. Spectrophotometric Characteristics of Blood Plasma with Varying Indole Content. 1, Control; 2, 6.25 mg %; 3, 1.25 mg %; 4, 0.31 mg %.

In view of the fact that concentrations of indole larger than 0.009-0.01 mg/l cannot be made, we conducted tests on five rabbits with intravenous administration of indole in order to study the rate at which indole is rendered harmless in an organism. For this purpose 10 mg of alcohol solution of indole were administered to a rabbit through an outer vein of the ear. We took blood samples for analysis from the outer vein of the other ear fifteen minutes, forty-five minutes and one hour and forty-five minutes following the administration. We detected about 0.3 mg % of indole in all of the rabbits in the first portion /194 of the blood taken after fifteen minutes. We did not notice any indole in the blood taken after forty-five minutes and after one hour and forty-five minutes. Parallel investigations of the amount of indican in the blood showed that the amount of it after administration of indole in the blood did not increase. Evidently, the indole which was administered not only was quickly rendered harmless but also was removed from the organism of the animals. A direct test which would indicate the rate of removal of indole from an organism would be a determination of the indican not only in the blood but also in the urine. However, we were not able to obtain urine by means of catheterization of the bladder in the rabbits at the same time period at which the blood was removed.

Taking into account that indole is quickly rendered harmless and removed from the organism, its unpleasant odor can be considered a principal hygienically significant property. We conducted investigations with the participation of fourteen volunteer test subjects in order to determine the threshold of perception of the odor of indole by man under laboratory conditions. The test subject was placed behind a screen, through which a rubber hose was extended, which ended in a glass bulb. The bulb inserted in the nose.

The initial concentration was distributed by the aid of a Zhanne syringe in the necessary amount one time, and a sweet-smelling mixture was fed through the bulb into the nose with a volume of 100 mℓ at the moment of inspiration. Feeding of the sweet-smelling mixture alternated with feeding of pure air. Repeated investigations were carried out with the test subjects no earlier than after two days. We made recordings of the rate of respiration in addition to determination of the threshold of perception of the odor on the "Al'var" electroencephalograph, the EKG with the second standard lead and the EEG in bipolar recording "forehead – back of the head." Perception of the indole odor by man was as follows:

Concentration, mg/m^3	0.45	0.9	1.12
Number of test subjects	12	13	12
Persons who had a positive response	4	12	6

As can be seen from the data presented four test subjects out of twelve sensed the indole odor at a concentration of 0.45 mg/m^3. The absolute majority of the test subjects clearly sensed the indole odor beginning at a concentration of 0.9 mg/m^3. A concentration of 0.45 mg/m^3 should be considered the threshold for perception of the indole odor by man.

Substantial changes in the recorded physiological indices due to the effect of indole did not appear in concentrations below 0.12 mg/m^3. During the action of higher concentrations, immediately following the feeding of a stimulant, two to three inhalations were nonuniform. The nonuniformity of respiration occurred during the action of detected concentrations and evidently was a response to stimulation of the olfactory analyzer. We did not detect any changes on the part of the EKG and EEG during the action of the indole vapors /195
in a concentration up to 9.0 mg/m^3. Subjectively the test subjects noticed a very unfavorable odor in concentrations approaching 0.9 mg/m^3. Two of them complained of nausea.

Thus, during the one-time inhalation of indole vapors in concentrations of up to 9.0 mg/m^3 no essential changes occurred in the organism. However, the unfavorable odor of indole caused negative subjective sensations and some reflex reaction.

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PROBLEM OF TOXICITY OF EXHALED AIR

V. V. Kustov, L. T. Poddubnaya and V. I. Mikhaylov

ABSTRACT: Peculiarities of the biological action of air, exhaled by man, in tests on white mice were studied. It was determined that a group of gasious toxic substances which are contained in exhaled air cause neural humoral shifts in an organism which involve a certain intensification of the inhibiting reaction in the nervous system.

The toxic action of exhaled air was first discovered in a series of works /196
by Brown-Sequard and d'Arsonval, 1887, 1887a, 1888). These works gave rise to numerous investigations, whose authors studied the peculiarities of the biological action of chemical substances which were unidentified at that time in their tests of isolated frog hearts (Ghandr, 1897; Gramenitskiy, Sivertsev, 1935; Pavlovskiy, 1937; Lehmann, Jessen, 1890; Peters, 1906; Lange, 1914 and others).

The results of the completed investigation seemed contradictory, and in experiments with laboratory animals which had been placed in ventilation chambers connected in series, the authors were not able to confirm the data of Brown-Sequard and d'Arsonval on the harmful action of air exhaled by animals on an organism (Zhandr, 1897; Formanek, 1900; Lehmann, 1919).

In experiments carried out jointly with M. M. Korotayev, G. I. Meleshko and Ye. Ya. Shepelev (Korotayev et al., 1967) we established that the exhaled air of healthy persons caused a noticeable suppression of chlorella photosynthesis when inserted into the air flow of a photosynthetic gas exchanger.

Therefore we deemed it wise to continue our study of the toxic action of air exhaled by man in tests on laboratory animals.

We conducted our studies on white male mice weighing 20 to 22 g. We collected air exhaled by man in Douglas bags and, after diluting it with medical oxygen (up to 20.5%) over a period of two hours we passed it through an inoculation chamber with the animals positioned inside. In order to eliminate the toxic action of the carbon dioxide gas which reached a

concentration of 3.3% in the exhaled air, we set up a control series of experiments in which the animals were subjected for two hours to the influence of a specially prepared nitrogen-oxygen mixture which contained 20.5% of O_2 and 3-3.2% of CO_2.

The biological action of the air exhaled by man according to the general condition and behavior of the animals and according to its effect on the neuro-humoral actions of the organisms was evaluated, for which purpose it was /197
recorded the cholinesterase activity of the blood of the experimental mice by the A. A. Pokrovskiy method (1953) and the amount of the acetylcholine by the Khestrin method using the L. Ya. Livshits and V. I. Rubin modification (1961).

A determination of the carboxyhemoglobin level in the blood of the white mice by the Vol'f method (Teysinger et al., 1959) and the catalase activity of the blood using the A. N. Bakh and S. R. Zubkova method (1950) before the test and after its completion in addition to the enumerated biochemical indices was made.

The experiments which we conducted showed that even though the nitrogen--oxygen mixture did not cause substantial changes in the general condition and behavior of the experimental animals the white mice did become sluggish, immobile, and sleepy following the two-hour action of the exhaled air diluted by oxygen.

Upon completion of the tests the level of the carboxyhemoglobin in the blood and its catalase activity (Figure 1) in the experimental mice were significantly higher than in the control group of animals. There was a similar change in the cholinesterase activity of the blood and its acetylcholine concentration (Figure 2).

The amount of certain gas-like toxic substances in exhaled air which /198
enters the inoculation chamber (average from five determinations) is given below:

Ammonia and aliphatic amines	0.0011 mg/l
Acetone	0.0008 mg/l
Organic acids in conversion to acetic acid	0.057 mg/l
Carbon monoxide	0.003 mg/l

Carbon dioxide	3.3%
Oxygen (after dilution of the mixture)	20.5%
General oxidizability of the exhaled air	0.106 mg O_2.

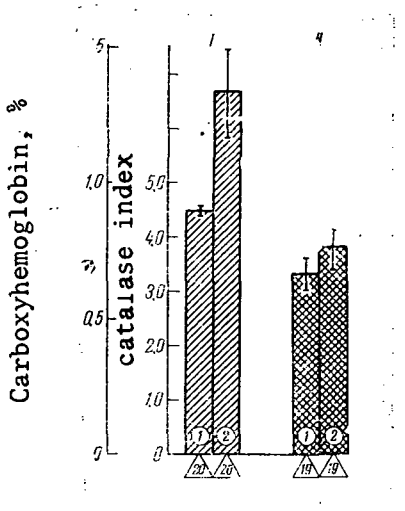

Figure 1. Effect of Toxic Substances Contained in Exhaled Air on the Level of the Carboxyhemoglobin (1) and Catalase Activity (II) of the Blood of White Mice. 1, Control; 2, Test; In the triangle, number of animals.

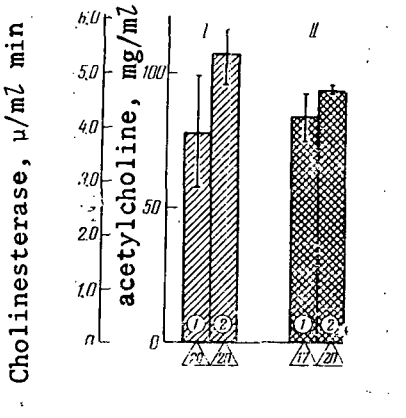

Figure 2. Effect of Toxic Substances Contained in Exhaled Air on the Cholinesterase Activity (I) and the Amount of Acetylcholine (II) in the Blood of White Mice. Designations are the same as in Figure 1.

If we compare the clinical picture of poisoning of animals by exhaled air to shifts in the acetylcholine-cholinesterase system, we can conclude that this action leads to impairment of the neuro-humoral relationship in the organism of white mice which can promote some accumulation of acetylcholine in the organism which would lead to intensification of the inhibiting reactions in the nervous system. The physiological significance of these reactions during the short-term action of exhaled air is evidently not very great. However, their introduction enables us to suppose that the more or less prolonged action of toxic substances contained in exhaled air of healthy persons may have some effect on the organisms.

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V. LIFE SUPPORT SYSTEMS FOR THE CABINS OF SPACE VEHICLES

PECULIARITIES OF DETERMINING THE OXIDIZABILITY OF WATER DURING ITS REGENERATION

V. A. Kryuchkov and N. S. Mareyeva

ABSTRACT: The completeness of oxidation of organic impurities in the condensate of the atmospheric vapors of an inhabited cabin was studied. All of the errors which were determined were analyzed. A conclusion of the possibility of using a different method of determination of oxidizability for the analysis of the regenerated water was provided. These methods have been developed for ground waters, stagnant waters and other waters.

Evaluation of the degree of contamination of water by organic impurities /199
can be conducted by determining its oxidizability. By studying processes for the regeneration of water from liquid products of the vital functions of man it was determined that the permanganate and bichromate oxidizability of solutions according to different methods which are recommended in the literature (Drachev et al., 1953; Kul'skiy, 1963; Bakhman et al., 1960; Savchenko et al., 1961; Lur'ye et al., 1962 and others).

Inasmuch as determination of the oxidizability is based on permanganatometry and chromatometry, i. e., quantitative methods of volumetric chemical analysis, this index must also quantitatively reflect the contamination of water by different impurities which are capable of oxidation. For example, the oxidizability of water which contains 170 milligrams/liter of ethyl alcohol must coincide (within limits of measurement error) with the calculated value of 118 milligrams O_2/liter during oxidation of ethanol to acetic acid and 354 mg O_2/liter during oxidation to carbon dioxide. Lowes oxidizability values would have indicated incomplete oxidation of ethyl alcohol, while exceeding the indicated values would have been proof of other compounds besides ethyl alcohol in the water.

In our investigation to check the authenticity of the results obtained in determining the oxidizability of regenerated water we simultaneously determined the oxidizability of solutions of ethyl alcohol.

Table 1 shows the results of a determination of oxidizability of water which we obtained in the process of a multistage purification of the condensate of atmospheric vapor in an inhabited hermetically-sealed cabin. As a result of the preliminary analysis of a specimen we quantitatively determined the amount of ethyl alcohol. Its concentration was 170 mg/ℓ. According to the /200
results of determination of the oxidizability, we intended to establish the presence and approximate concentration of the remaining unidentified impurities. However, drawing any conclusion as to the degree of contamination of the water on the basis of the data presented in the table which differ by more than an order of magnitude, is impossible.

An analysis of the results obtained and the additional experiments which we carried out showed that the difference noted in the table in the degree of oxidizability of water samples was due to the following factors:

--losses of volatile impurities and water vapor during boiling of the sample in an open vessel (a flask covered with a funnel);

--incomplete oxidation of impurities which contaminate water in a 5 to 10 minute interval, added to the reaction according to the methods of Kubyel', Shul'tse-Papp and Tyurin;

--automatic decomposition of the permanganate when the sample is boiling;

--difference in activity of oxidizing agent.

Losses of volatile substances along with water vapor are more clearly reflected in the values of oxidizability during preliminary evaporation of the sample according to the method of Tyurin (Table 1, Item 5). The oxidizability of the condensate which was determined according to this method is /201
9 mg O_2/ℓ, which reflects the concentration of only a small amount of volatile impurities in the water. The solution of ethanol in this experiment has zero oxidizability .

Boiling of a water sample whose contamination is determined basically by volatile substances also involves certain losses of components if it is conducted in a vessel covered with a funnel (Table 1, Items 1, 2, 4). This shortcoming was eliminated in a method recommended by agencies of the Water

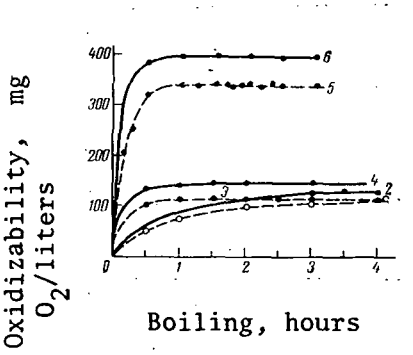
Administration of the Council for Mutual Economic Assistance (CEMA) and the World Health Organization (WHO), according to which boiling the sample is carried out in a flask equipped with a reflux condenser (Table 1, Item 6).

TABLE 1. OXIDIZABILITY OF A CONDENSATE OF ATMOSPHERIC MOISTURE AND A SOLUTION OF ETHYL ALCOHOL (170 Mg/Liters), DETERMINED BY DIFFERENT METHODS.

Method of determination	Literature source	Oxidizability, milligrams O_2/liters	
		Condensate	Solution of ethanol
1. Titration by a solution of potassium permanganate in an acidic medium during heating (Kubel' method)	GOST 4495-49 (Drachev et al., 1953 and others)	28	11
2. Titration by potassium permanganate in an alkaline medium (method of Shul'tse-Papp	The same.	29	12
3. 4-hour oxidation by potassium permanganate in acidic medium with a temperature of +28°	(Unified methods for study of water quality. SED Press, 1965)	131	118
4. Bichromate oxidizability with boiling of a sample for 5 min. (Tyurin method with Komarova modifications)	(Bakhman et al., 1960; Savchenko et al., 1961)	99	90
5. Bichromate oxidizability with evaporation of water sample (according to Tyurin)	(Drachev et al., 1953; Kul'skiy, 1963)	9	0
6. Bichromate oxidizability with 2-hour boiling of the sample with addition of 50% sulfuric acid (recommendation of water conservation organs of CEMA and WHO	Unified methods for studying the quality of water. CEMA Press, International Standards for drinking water. WHO Press, 1964)	402	333
7. Bichromate oxidizability during 2-hour heating of a sample on a boiling water bath	Op cit.	147	118

Translator's note: GOST = State Standard.

Another essential element of the method for determining the oxidizability is the length of the reaction which we studied relative to different methods of oxidation (Figure).



Kinetics of Oxidation for a Weak Solution (170 m/ℓ) of ethyl alcohol (Broken Line) and a Condensate of Atmospheric Moisture with the Same Amount of Ethanol (Solid Line). 1, 2, Oxidation by permanganate according to the CEMA method; 3, 4, Oxidation by bichromate on a boiling water bath; 5, 6, Oxidation by bichromate according to the method of CEMA and WHO.

It follows from the figure that under conditions of boiling the sample, the duration for complete oxidation of the impurities is computed in tens of minutes. Consequently for the five-minute period of time used for reaction, according to the method of Tyurin-Komarova only a part of the impurities can be oxidized.

In contradiction to the latter, one-to-two-hour boiling of the sample which is recommended according to the method of CEMA and WHO, ensures that all the oxidation reactions which are possible in the chromate medium used will occur.

The third factor which distorts results in determination of the oxidizability of water is the spontaneous decomposition of the potassium permanganate during boiling of the sample, whose mechanism has been described by Kol'tgof (1932, 1961).

Spontaneous decomposition of the potassium permanganate while boiling in the samples of water which we studied occurs quite a bit faster than in the control with still water. A consequence of this process is an overestimation of the results of determination of the oxidizability of the samples. This overestimation of results is not evident in analysis carried out according to the method of GOST 4495-49 (Tables 1, Items 1, 2), but is hidden by the prevailing /202

effect of the first two factors: the losses and incomplete oxidation of the impurities.

In considering the effect of the enumerated three factors, one should include that the value of the permanganate oxidizability of the regenerated water which is determined by the method of State Standard (GOST) gives substantially lowered results when this systematic error is not determined (Table 1, Items 1, 2) and therefore cannot be a quantitative criterion of the amount of axidizable impurities in the water.

According to the data of the literature, the effect of spontaneous decomposition of the potassium permanganate can be significantly reduced by conducting the oxidation reaction at room temperature (Kol'tgof, 1932, 1961). The method for determining the permanganate oxidizability of stagnant water by a four-hour oxidation at room temperature of +28°, which is recommended by the Conference of Administrators of Water-Control Agencies of the CEMA, satisfies this condition to a great.extent.

The drawings show the kinetics of the oxidation by permanganate of a solution of ethyl alcohol (170 mg/l) and of a condensate of atmospheric vapor which contains the same amount of ethanol. Oxidation was carried out according to the CEMA method. In conformity with the kinetic curves, the oxidation--reduction reactions proceed for three to four hours. Substantial decomposition of the permanganate does not occur afterward. The oxygen requirement of the solution of ethyl alcohol is 118 mg O_2/l, which corresponds to complete oxidation of ethanol to acetic acid. Oxygen consumption during oxidation of the condensate of atmospheric moisture is 131 mg O_2/l. Exceeding the oxidizability of the condensate in comparison with the solution of ethanol by 13 mg of O_2/l confirms the presence in its composition of a small amount of unidentified impurities. The presence in the composition of the condensate of a number of impurities was supported by methods of volumetric chemical analysis.

In addition to the above-mentioned effect of the three factors which leads to obvious errors of a methodological nature, the value of the oxidizability

of the examined water sample depends on the activity of the oxidizing agent. The activity of the permanganate and, especially, the bichromate, which is determined by the oxidizing potentials depends on the acidity of the medium to a significant degree. The relationship of the real potentials of the system $Cr_2O_7^{2-}/Cr^{3+}$ to the amount of sulfuric acid in a solution has been introduced by Kol'tgof (1932, 1961).

In Table 2 we have presented the oxidizability of the solution of ethyl alcohol (that is, of one of the possible impurities in the regenerated water), which depends on the acidicty of the medium. Oxidation of the samples was carried out according to the methodology of Tyurin-Komarova with the following modifications: the boiling time of the samples was 2 hours (with a reflux condenser); the amount of sulfuric acid added to the sample varied. /203

TABLE 2. BICHROMATE OXIDIZABILITY OF A SOLUTION OF ETHYL ALCOHOL (100 mg/liter) IN RELATION TO THE AMOUNT OF SULFURIC ACID IN THE SAMPLE.

No. of test	Amount of H_2SO_4 in the sample		Oxidizability of solution, mg O_2/liter	
	% by volume	% by weight	During boiling of solution for 2 hours	During heating of the sample in a boiling water bath for 2 hours
1	8.7	14.6	69	70
2	10.2	17.1	70	70
3	12.5	20.4	69	69
4	16.0	25.6	80	69
5	19.2	30.0	82	70
6	22.2	34.0	86	69
7	34.3	48.6	128	69
8	40	59.3	160	70
9	47.5	62.1	195	69
10	50	64.4	197	70
11	60	73.4	196	--

From Table 2 it follows that in adding 8.7-12.5% of sulfuric acid to the sample (i.e., a concentration suggested in the methodology of Tyurin-Komarova) the oxidizability of the solution is 69-70 mg O_2/liter, which corresponds to the oxygen consumption during oxidation of ethyl alcohol to acetic acid according to the reaction:

$$3C_2H_5OH + 2K_2Cr_2O_7 + 8H_2SO_4 \rightarrow 3CH_3COOH + 2K_2SO_4 + 2Cr_2(SO_4)3 + 11H_2O.$$

The same degree of oxidation of ethanol is observed in a wider range of sulfuric acid concentrations when the sample is heated on a boiling water bath. In this case the resultant data on oxidizability are more stable.

Boiling a sample with a high amount of sulfuric acid leads to an increase of oxygen consumption which indicates continued oxidation of the primary reaction products. When 47.5-60% sulfuric acid is added to the sample, its oxidizability is equal to 195-197 mg O_2/liter. This corresponds to 94-95% of the oxygen consumption necessary for oxidation of ethanol to carbon dioxide according to the reaction:

$$C_2H_5OH + 2K_2Cr_2O_7 + 8H_2SO_4 \rightarrow 2CO_2 + 2K_2SO_4 + 2Cr_2(SO_4)3 + 11H_2O.$$

The high oxidizing potential of the concentrated sulfur-chrome medium, the high boiling temperature of the samples, and the use of a catalyst create conditions for complete oxidation of almost all the organic compounds to carbon dioxide. This property of the sulfur-chrome medium is used in a method for determining the bichromate oxidizability of water which is recommended by agencies of CEMA and WHO. /20

The high degree of oxidation of the impurities is explained by the high values of oxidizability of the condensate and the alcohol solution, which are determined according to the given method (see Table 1, Item 6). If four gram-equivalents of bichromate are used up during complete oxidation of one mole of ethyl alcohol to acetic acid under "soft" conditions, then three times as much potassium bichromate is used up during its complete oxidation to carbon dioxide under "hard" conditions.

From the drawing it follows that oxidation of ethyl alcohol and other impurities using the method of CEMA and WHO is completed during the first 30 minutes of boiling of the sample. Longer heating does not bring about a reduction in the concentration of bichromate in the solution, which is evidence of its stability in the medium and of the completion of all possible oxidation reduction reactions.

From the curves of the oxidation kinetics it also follows that the oxidizability of the condensate is 402 mg O_2/l and the oxidizability of distilled water containing the same amount of ethyl alcohol is only 333 mg O_2/l. The difference between these values, 69 mg O_2/l, reflects the contamination of the condensate by other compounds.

The method recommended by agencies of WHO and CEMA allows us to detect many chemical compounds in the regenerated water which do not oxidize in the "softer" conditions as, for example, during oxidation of potassium permanganate for four hours.

Along with the great advantages, the method has one essential shortcoming which is revealed during oxidation of solutions which contain ethyl alcohol: the latter oxidizes to carbon dioxide by less than 92-96%. Oxidation of the remaining amount of ethanol evidently occurs only as far as carbon monoxide. The degree of oxidation within the limits of the values introduced in each specific test depends on random uncontrollable factors. This introduces the well-known difficulties in studying the processes of regeneration of water and requires numerous repetitions in determination of oxidizability for calculation of mean results.

In this connection, the method using a boiling water bath for heating the examined samples with chrome mixture and a catalyst deserves attention. In this instance the results of bichromate oxidizability which we obtained are distinguished by their great stability. The extent of oxidation of the mixtures and, possibly, the number of reacting compounds at 100°, are of course lower than during boiling of a concentrated sulfur-chrome mixture. However, these /205
indices are higher than during oxidation of a permanganate sample for 4 hours.

Further studies have shown that regularities noted in determinations of the oxidizability of a condensate of atmospheric moisture are also present during analyses of the condensate obtained during distillation of urine.

Conclusion

Water which is regenerated from the products of human vital functions differ sharply in composition of possible impurities from surface and ground waters which serve as the source of ordinary water supplies, which requires a different approach in selecting a method for determination of its oxidizability.

Only those data on oxidizability which correspond to necessary and adequate oxygen consumption for completing all oxidation-reduction reactions which are possible under conditions used for analysis of media may serve as a quantitative index for evaluation of the degree of purification of regenerated water. Of the methods studied by us for quantitative determination of the oxidizability of regenerated water, the following may be used: the bichromate method recommended by CEMA and WHO; the four-hour oxidation permanganate method recommended by CEMA. The method for determination of the bichromate oxidizability of CEMA and WHO may be used with the following modification: heating the water sample with the oxidant with sulfuric acid and with silver sulfate is carried out in a water bath in a flask with a reflux condenser for 1-2 hours.

In evaluating the results of the analysis, one must consider the degree of oxidation of the principal mixtures used as oxidizing media.

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STUDY OF THE TECHNOLOGY OF DECONTAMINATION OF WATER WHICH HAS BEEN REGENERATED FROM THE LIQUID PRODUCTS OF HUMAN VITAL FUNCTIONS

V. A. Kryuchkov and L. I. Rogatina

ABSTRACT: Studies were conducted on the technology of decontamination of water during its regeneration from products of the vital functions of man. It has been shown that the starting products may contain a large quantity of microflora. In order to guarantee decontamination of the regenerated water the following set of technological procedures have been developed: conservation of urine, filtration of condensate of water through sorbents, preparation of components of a system from materials which have antimicrobial properties.

The principal task of a technological process for regeneration is the /206
recovery of water which is suitable for food purposes from the products of vital functions of man, i.e., water which does not contain mixtures which are harmful or undesirable. In addition the regenerated water should not contain disease-causing bacteria. Because even a simplified bacteriological analysis during spaceflight is quite difficult, the technology for regeneration of water should include elements which guarantee its decontamination.

In the literature, only certain data on decontamination of the regenerated water have been published. Hendel (1962) noted in a review of methods of regeneration that there is a large quantity of bacteria in the distillate during vacuum distillation of urine; in order to decontaminate distillates he suggests that they be filtered through a bactericidal filter. The decontaminating role of a catalytic oven (Konikoff and Reynolds, 1963) was noted in a study of the process of urine distillation with high temperature catalytic oxidation of the volatile substances. A large quantity of microflora was discovered in water which had been condensed from the atmosphere of an inhabited cabin during investigation using a multifilter system for regeneration. In order to decontaminate the condensate the authors also suggested use of a filter which would trap microorganisms (Wallman et al., 1965).

We carried out studies of the technology of decontamination of water in tests of regenerating units which we conducted for several days. The regenerating units were installed on test stands and in a hermetically sealed chamber.

We used known methods for bacteriological studies (Kalinina, 1966) in studying the microflora of the initial products, the intermediate products and the regenerated water.

Naturally, the amount of microorganisms in the regenerated water depends on two factors: contamination of the starting products (urine and atmospheric moisture) and the action of physical processes, chemical reagents and materials, to which these starting products are subjected during the recovery and purification of water.

Different data on quantitative and qualitative composition of microflora in the urine of healthy persons are reported in the literature. These variations are quite significant (Novikov, 1939; Philpot, 1956; Calvin, 1961). /207

Functional changes in the urogenital system and, related to this, the onset of bacteriuria can be expected during spaceflights.

The recently eliminated urine which was used in the test data had a contamination in the vicinity of 200-400 colonies per ml.

We discovered white and golden staphyloccus in a composition of the microflora. There were no pathogenic microorganisms in the examples tested. However, in devising regenerating systems one should remember that there may be latent bacilli carriers in the environment of healthy persons.

Urine is a favorable medium for the vital functioning of microorganisms and during storage its initial contamination quickly increases. After only a few hours, progressive increase of microflora is observed at room temperature. After twenty-four hours, the number of microbes in 1 cm of urine may reach many millions. Urine is intensively decomposed by microbes with separation of a large amount of ammonia, carbon dioxide and foul-smelling substances; the pH increases to 9-10. The increase in the alkalinity of the fluid has a depressing effect on the microflora. The amount decreases and processes of decomposition of the organic components of the urine later stabilize.

At the present time the most likely method for regeneration of water from urine is its evaporation, carried out technologically according to different variants (distillation at atmospheric pressure, vacuum distillation, evaporation in an airstream, etc.), with subsequent final clarification of the water condensate on sorbents. Distillation of the urine at a temperature higher than 100° is accompanied by its decontamination. During vacuum distillation and during evaporation in a stream of air, evaporation of water from urine normally proceeds at room temperature or at a temperature near room temperature. In this case entry of microorganisms into the condensing water is unavoidable. Microflora are carried to the condenser from the evaporator in the airstream or in water vapors contained in aerosols. Of course, during weightlessness the probability of microfloral accumulation in the condenser increases.

One procedure which protects the water condensate from contamination by microorganisms is the conservation of the original urine, which is preferably brought in at the beginning of the line, that is, in the urine receptacle. At the present time many preserving agents have been developed which obstruct the development of microflora in the urine or completely sterilize it (Putnam, 1965). Phenols, toluene, different oxidants, quaternary ammonia compounds, and salts of heavy metals belong to this group. The conservant used should be compatible with the general technology for regeneration, that is, the /20
conservant and the products of its reaction with the urine and materials should be either nonvolatile or should be easily adsorbed by sorbents which are used for final purification of the water condensate.

According to our data, the conservation of the original urine even has a positive effect on the chemical composition of the distilled water by lowering its contents of ammonia and foul-smelling substances.

Atmospheric moisture is the second source of "raw material" for making drinking water. Studies have shown that water which is condensed from the atmosphere of a manned cabin is always contaminated by microorganisms which enter it from the stream of cooled air along with dust and aerosols.

The degree of contamination of the atmospheric moisture condensate bears a direct relationship to the amount of microorganisms found in the air of a manned cabin and is determined by many factors among which the most important are: effectiveness of removal of the microflora from the air during its regeneration, the general sanitary condition of the hermetically sealed cabin, of the linen and equipment of the crew, including the effectiveness of the preliminary disinfection process, and the duration of the crew's stay in the hermetically sealed cabin, etc.

In one of our tests we found a relationship between the amount of microflora in atmospheric moisture condensate and the duration of stay of two researchers in a hermetically sealed chamber with a volume of 5 m^3. Water vapor condensation was carried out in an experiment on previously disinfected and cleansed Duralumin surface:

Length of stay in hermetically sealed cabin, days.....	4	6	12	13
Amount of microorganisms in 1 ml of condensate.....	$8.5 \cdot 10^3$	$4 \cdot 10^3$	$4.9 \cdot 10^4$	$9.6 \cdot 10^5$

An analysis of the microflora showed the presence of the following types of microorganisms in the condensate: white and golden staphylococci, yellow sarcine and various kinds of mold. There were significantly fewer hemolytic strains of microflora in the condensate in comparison to the microflora of the air, which might be explained by the degree of antagonism of microorganisms in a liquid medium.

The recorded amount of contamination in the condensate is not the limit, for in a number of other tests it was higher. Table 1 shows the amount of microflora in water obtained in a joint condensation of vapor of atmospheric moisture and water vapor which was formed during evaporation of urine in an airstream at room temperature. Conservation of urine was carried out in a test with sulfuric acid and hydrogen peroxide. However, complete halting of the growth of microflora in the urine was not achieved because of the insufficient mixing of the preserving components. The amount of bacteria in 1 ml of united condensate reached a value of $1.3 \cdot 10^6$ /209

Further intensive growth of bacteria was not noticed during storage of condensate from atmospheric moisture and water condensate obtained from the urine.

TABLE 1. AMOUNT OF MICROORGANISMS IN 1 mℓ OF WATER CONDENSATE BEFORE AND AFTER ITS FILTRATION THROUGH SORBENTS.

Duration of experiment, days	Amount of microorganisms		Amount of condensate filtered from 1 volume of filter gas absorber	Effectiveness of purification, %
	Before filtration	After filtration		
4	280	0	3.5	100
6	480	0	7.0	100
7	3,250	0	10.8	100
10	$1.2 \cdot 10^5$	2	12.8	99.99
14	$2 \cdot 10^5$	3	18.3	99.99
18	$1.5 \cdot 10^5$	33	30.8	99.98
20	$4 \cdot 10^5$	400	33.8	99.90
22	$1.3 \cdot 10^6$	480	36.0	99.97

In conformity with the technology of regeneration we carried out purification of the water condensate by means of filtration through ion exchange resins and activated charcoal.

It is known that ion exchange resins and activated charcoal are capable of absorbing bacteria found in water in addition to absorption of chemical compounds. Table 2 shows results of a microbiological analysis of a mixed condensate after its filtration through purification filters.

The gas absorber of the filters consisted of a strongly acidic cation exchanger, a medium basic anion exchanger, a strongly basic anion exchanger and activated charcoal. The useful volume of the filters was 1,800 mℓ and

provided removal of 43 l of harmful or undesirable impurities from the condensate. In addition, the filters also absorbed microorganisms. The effectiveness of removal of microflora from the water was 99.9% which assured its decontamination according to the standards of GOST, with the exception of extreme cases of very high original contamination.

In the next experiment, whose purpose was to lower the original contamination of the condensate, some parts of the heat exchanger on which condensation of water vapor occurs and which were formerly made of an aluminum alloy were replaced by brass.

The decontaminating action of preparations of heavy metals in solutions /210
is generally known. Use of parts made of heavy metals in a regenerating system has the advantage that the necessity of a special device for supplying preservatives to the unit is removed.

Table 2 gives the results of a bacteriological analysis of the atmospheric moisture condensed on a brass surface, obtained in a number of experiments lasting from three to sixty days.

TABLE 2. RESULTS OF CHEMICAL AND BACTERIOLOGICAL STUDIES OF ATMOSPHERIC MOISTURE CONDENSED ON A BRASS SURFACE AND SUBSEQUENT FILTRATION THROUGH SORBENTS.

Duration of experiment, days	Amounts in the starting condensate			Amount in the condensate after filtration through sorbents		
	Ammonia, mg/l	Copper, mg/l	Colonies of microorganisms in 1 ml	Ammonia, mg/l	Copper, mg/l	Colonies of microorganisms in 1 ml
1	4.0	1.0	0	0	0	0
10	6.0	1.4	0	0	0	0
14	10	2.2	0	0	0	0
3	18	3.2	0	0	0	0
30	31	6.0	0	0	0	0
60	25	4.0	0	0	0	0

In the batches of condensate which we tested the amount of copper was 1 to 6 mg/l and depended not on the duration of the experiment but on the concentration of ammonia. This indicates the active influence of ammonia on the process of dissolution of a brass surface by means of the formation of ammonia complex compounds of copper.

Microbiological studies show that a condensate which contains 1.0 mg/l and more of copper is sterilized. Sterility of the condensate is preserved even after its filtration through sorbent and also during subsequent prolonged storage.

Copper ions are completely absorbed by ion exchange resins during filtration of the condensate through sorbents. Two-to-four mg of ion exchange resin are needed for absorption of 1 mg of copper which becomes a fraction of a percent of the overall requirement of sorbents for removal of all other impurities from the condensate.

Studies of the decontamination of the condensate through its contact with parts of the system which are made of silver yielded less positive results because the surface of the latter became covered by a nonsoluble carbonate or chloride film in the course of the experiment.

On the basis of our data and those of sources in the literature we concluded that in order to prevent growth of microflora in apparatus and vessels of the water regenerating system, and also to guarantee decontamination of water which has been regenerated from liquid waste products of human vital functions, the following set of measures are effective: /211

--conservation of recently eliminated urine by chemical reagents; e.g., by ions of copper, sulfuric acid, boric acid, hydrogen peroxide, etc.;

--use of ion exchange resins and activated charcoal to reduce the microbial contamination of the condensed water;

--the manufacture of certain parts of the system with copper or other materials which have antimicrobial properties in a water medium in order to suppress the growth of microflora during regeneration of the water.

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EFFECT OF THE FREQUENCY OF CHANGING THE NUTRIENT SOLUTION ON PRODUCTIVITY OF PLANTS CULTIVATED ON KERAMZIT[1]

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ABSTRACT: This paper gives the results of a study of different time intervals for changing the nutrient solution in order to ensure its regeneration by the conservation of higher plants using the hydroponic method with a keramzit substrate. Experimental data show that in order for the greenhouse to function at the given productivity within limits of a calculated theoretical data, the nutrient solution must be changed once in 90 days. Later use of the solution leads to an increase in the area needed by the greenhouse. The necessity of frequent regeneration of large amounts of nutrient solution and the high absorbing capacity of the substrate make possible its use in greenhouses with closed cycles.

The development of methods for soil-free cultivation of plants which are /211
the principle determinants of the weight features of a greenhouse is necessary during construction of greenhouses with closed cycle systems. At the present time there are nonsubstrate methods of cultivation (air, water and film crops) /212
and substrate methods (hydroponics with different fillers).

The substrate methods of cultivation of plants are characterized by the presence of large volumes of nutrient solution (60-100 ℓ to 1 m^2 of sowing area) and substrate. The presence of a large volume of nutrient solution increases the strain on the regenerating units. Therefore it is important to determine the probable periods for use of nutrient solutions for cultivation of plants.

The aim of the present study is the determination of time periods for the exchange and regeneration of nutrient solution for cultivation of higher plants as exemplified by the hydroponic method with a substrate of keramzit. The criterion of the possible duration for use of the solution was the productivity of plants in joint cultivation which had to provide a specific portion of the ration of the cosmonauts for each unit of area.

[1]"Keramzit" - porous concrete aggregate [translator's note].

The question of time intervals for the exchange of nutrient solutions in hydroponic systems is quite inconsistent. There are a number of recommendations which say that the exchange of the nutrient solution must take place once in thirty days (Aliyev, 1965; Lychkin, 1965), once in sixty days (Bentley, 1965), once in sixty to ninety days (Maginitskiy, 1965); with a volume of nutrient solution of 60-80 l/m^2.

Certain researchers (Davtyan, 1965) recommend that the substrate be washed with pure water once in ten days without changing the solution in order to transfer a significant amount of the salts absorbed by the substrate into the nutrient solution and to reduce the accumulation of salts in the medium. In general, "...the problem of time intervals for the exchange of the nutrient solution always causes arguments. Some figure that they should be exchanged once a week, some say once a month and another group says once in every six months or once every year. Experience in Glen Hazel shows that the solutions could be used for long times if they are periodically analyzed and brought up to the proper level" (quotation from Bentley, 1965).

Our studies were carried out in units with a sowing area of 1 m^2 with artificial illumination of plants by ZN-8 bulbs. Illumination on the leaves was maintained at 100-150 W/m^2. The length of the lighting period was 18 hours. The plants were cultivated by the hydroponic method with irrigation by the nutrient solution three times a day. Keramzit which had been previously disinfected and carefully washed in the amount of 120 kg/m^2 of the 0.5-1.5 cm fraction was used as a substrate. The amount of nutrient solution in all of the tests was 80 l/m^2. We used a Knop mixture for a base solution. Depending on the test, the solution was adjusted or blended.

During the experiments we checked the nutrient solution once every three days for concentration of the principal nutrient substances. At this time we /213 determined the amount of N_{NO_3} by the Granval'-Lyazh method, the amount of N_{NO_2} by the Griss method, the amount of N_{NH_4} by the Nessler method, phosphorus by the Denizhe method, potassium by the method of flame photometry. The pH of the solution was adjusted to 5.6-5.8 daily. We measured the loss of water

daily and we increased the level to the original in all of the tests. The object of the investigation was joint cultivation of the following plants: white-headed cabbage, Chinese cabbage (Khibin variety), Nantskaya carrots, Dunganskiy radishes, Bordeaux sugar beets, "White Juicy" tomatoes.

Caring for the plants (depth of planting, watering, adding water and tying up) during vegetation was the same in all the tests.

The duration of the tests was ninety days, therefore crops with a short growing time were planted twice (radishes and Chinese cabbage). The tests were carried out with two repetitions according to the following items:

--use of the nutrient solution for ten days followed by exchange. Nine exchanges of solution were used during the vegetative stage;

--use of the nutrient solution for thirty days followed by exchange. Three exchanges of solution were made during the vegetative stage;

--use of the nutrient solution for ninety days without exchange during the vegetative stage;

--use of the nutrient solution for 180 days, that is for the duration of two vegetative stages, the plants were cultivated in the same solution;

--the use of the nutrient solution for 270 days, that is, for the duration of three vegetative stages the plants were cultivated in the same solution;

--the use of the nutrient solution for 360 days, that is, for the duration of four vegetative stages the plants were cultivated in the same solution.

As we said earlier, the basic index of the possibility of prolonged use of the nutrient solution for cultivating plants in a keramzit was their productivity. Evaluation of the productivity included the yield of dry and wet substance and also the ratio of the economically useful part to the economically nonuseful portion. Because the area of each crop in the mixed planting was small it seemed best to us to use the yield data of the entire planting from one square meter. As can be seen from Table 1, increasing the time periods for the use of the nutrient solution led to a reduction in the yield of the economically useful and nonuseful parts and of the biomass as a whole. If we take the variant with an exchange of solution of once in

ten days as optimum, the yield of the planting in the variant with exchange of solution once in 360 days is reduced by 56% with respect to the yield of economically useful portion and by 34% with respect to yield of economically nonuseful part and by 48.7% with respect to the yield of the entire biomass. /214

TABLE 1. YIELD OF MOIST BIOMASS FROM 1 m^2 OF JOINT CULTIVATION.

Exchange rate of the solution, days	Economically valuable part		Economically non-valuable part		Entire Biomass	
	kg	%	kg	%	kg	%
10	22.7	100	11.9	100	34.6	100
30	18.5	81.5	11.1	93.3	29.6	85.8
90	17.9	78.8	8.6	72.3	26.5	76.6
180	15.7	69.1	8.0	67.2	23.7	68.5
270	11.4	50.4	7.6	63.9	19.0	54.9
360	9.9	43.6	7.9	66.3	17.8	51.3

The yield of the biomass in dry substance (Table 2) progressively decreases as the duration of the use of the nutrient solution increases. In addition, the portion of the economically nonuseful part increases from 0.73 to 1.14 in the variant with exchange of nutrient solution every ten days in comparison to the variant with exchange of solution every 360 days, which is indicative of the significant reduction of the economic effectiveness of the planting.

TABLE 2. YIELD OF DRY BIOMASS FROM 1 m^2 OF JOINT CULTIVATION OF CROPS

Exchange rate of the solution, days	Economically valuable part		Economically non-valuable part		Entire Biomass	
	grams	%	grams	%	grams	%
10	1,469.6	100	1,071.8	100	2,541.4	100
30	1,393.0	94.8	1,029.0	95.9	2,422.0	94.3
90	1,176.2	80.0	746.5	69.6	1,922.7	75.6
180	1,010.0	68.7	804.2	75.0	1,814.2	71.4
270	811.6	55.2	777.4	72.5	1,589.0	62.5
360	687.9	46.8	782.9	73.4	1,470.8	57.8

We monitored the nutrient solution for its concentration of nitrogen, phosphorus and potassium in the remaining solution. The loss of elements from the nutrient solution was determined according to the difference between the total amount of elements in the initial and remaining solutions and the loss was made up for by adjustments in the level of the Knop solution. Absorption /215 of elements for each time interval was cumulative.

As can be seen in Table 3, absorption of the principal elements of mineral nourishment during prolonged use of a nutrient solution (for 360 days) drops by 33.5% for nitrogen, by 40.9% for phosphorus, and by 40.6% for potassium, in spite of the fact that the concentration of these elements in the solution was maintained at one level the whole time.

TABLE 3. ABSORPTION OF N, P, K FOR 90 DAYS FROM 1 m^2 (IN GRAMS).

Rate of solution exchange, days	N	P	K	Ratio of N:P:K in the solution
10	122.0	70.8	170.8	1:0.58:1.40
30	150.1	89.1	202.7	1:0.60:1.35
90	100.3	84.1	169.9	1:0.84:1.69
180	80.7	62.2	97.6	1:0.78:1.27
270	74.6	61.1	109.5	1:0.82:1.40
360	80.7	41.5	100.9	1:0.51:1.25
% of absorption decrease in comparison to an optimum variant	33.5	40.9	40.6	--

Decrease of the absorption of elements of root nutrition and crop productivity may be caused by a number of factors. Among these may be root secretions of plants which have a specific effect, salinization of the substrate due to its high adsorption capacity or disaggregation of the substrate and washing of a number of elements into the solution which have a toxic effect.

We confirmed the high absorption capacity of the test substrate by the following test. We examined two variants: plants plus keramzit and keramzit without plants. Both variants were kept on similar schedules for feeding, illumination and water.

Absorption of the elements was determined according to the difference between the total amounts of initial and remaining solutions. The results of this test are presented in Table 4. If we assume that the absorption of elements by keramzit was the same in the variants with plants and without them, out of the total amount of elements absorbed from the nutrient solution during the vegetation stage, the principal amount falls to the share of the keramzit: 72.7% phosphorus, 68.8% nitrogen and 69% potassium.

TABLE 4. ABSORPTION OF N, P, K BY KERAMZIT IN 1 m^2 FOR 90 DAYS (IN GRAMS).

Element	Plants + keramzit	Keramzit
N	128.0	88.1
P	79.7	58.0
K	185.0	127.6

The plants absorbed a very small part of the elements: 31.2% of the nitrogen, 27.3% of the phosphorus and 31% of potassium. It could even be maintained that the feeding of the plants wholly depended on the sorptive properties of the substrate.

The term "absorption by keramzit" /216 is somewhat conditional and implies the total loss of elements to substrate sorption, vital functioning of the microflora and a number of other factors. Thus, the test showed the high absorptive capacity of the keramzit which had been mentioned by a number of other authors (Revut, 1965; Shtreys, 1965), who had observed impoverishment of a solution with respect to nutrient elements and especially phosphorus. The high adsorptive capacity of the keramzit (absorption of 60-70% of the elements) prevents one from taking the balance characteristics of the principal elements of the mineral feeding.

The experimental material which we have introduced shows that during hydroponic cultivation of plants on keramzit with prolonged use of nutrient solutions, a reduction in yield occurs which leads in turn to an increase in the area of the greenhouse which is necessary to ensure the ration for the humans.

We used a tentative set of vegetable crops which would ensure 60% of the carbohydrate ration for humans and was introduced by V. P. Dadykin in his pamphlet "Space Gardening" with his calculations of the area and productivity of the greenhouse as our theoretical premises. According to these data, the hothouse should provide the human diet with 49.17 grams per day (without potatoes) in dry foodstuffs. Calculation of the area was carried out according to the formula

$$S(m^2) = \frac{a \cdot 100 \cdot t}{e \cdot b},$$

where a is the calculated amount of dried foodstuff products (in grams), which would provide the given ration; t is the period of vegetation (in days); e is the percent of dry foodstuffs in the test; d is the yield of crops in the test (in kilograms/m^2). In order to calculate the area of the hothouse we must determine the yield of the plants (Table 5) and the percent of dry foodstuffs (Table 6).

TABLE 5. CROP YIELD (IN kg/m^2) FOR 90 DAYS.

Crop	Calculated	Exchange rate of the solution, days					
		10	30	90	180	270	360
White-headed cabbage	7.0	21.5	13.0	15.6	14.8	8.9	3.0
Chinese cabbage	22.5	27.9	28.2	19.6	13.2	19.0	19.2
Radishes	18.0	37.8	33.7	19.6	8.4	5.0	14.3
Carrots	8.0	20.1	20.6	19.0	17.6	11.7	15.0
Sugar beets	7.5	20.1	14.3	19.2	13.2	16.1	11.5
Tomatoes	12.5	23.2	19.4	19.2	19.0	10.9	10.7

Analysis of the crop yield data obtained in the test shows that the area /217
of the hothouse must be increased from 2.9 to 11 m^2 with a calculated area of 5.1 m^2 (Figure) depending on the length of time the nutrient solution is used.

Hence, there must be an exchange of nutrient solution no less than once every 90 days in order to provide a ration of plant food from the calculated area of the greenhouse.

TABLE 6. CONTENT OF DRY MATTER IN ECONOMICALLY USEFUL PART OF PLANTS (IN %).

Crop	Calculated	Exchange rate of the solution, days					
		10	30	90	180	270	360
White-headed cabbage	10.0	5.94	5.98	4.65	4.47	5.08	5.52
Chinese cabbage	5.32	5.12	5.01	4.74	4.71	5.50	5.05
Radishes	6.50	5.83	6.19	4.90	6.57	6.12	6.08
Carrots	11.50	12.47	13.22	12.0	14.05	10.97	11.06
Sugar beets	14.0	8.71	8.62	13.21	12.01	10.17	10.36
Tomatoes	6.0	5.45	5.75	5.01	4.36	4.91	4.70

These data show that the area of the greenhouse can be reduced but in this case the frequency of exchange of the nutrient solution must be increased which undoubtedly would put an additional load on the regenerating unit for the nutrient solution.

All of the foregoing data lead us to believe that cultivating plants by prolonged use of a nutrient solution on a keramzit lowers the yield of the biomass per unit area, and the necessity of regenerating the nutrient solution /218 and the high adsorptive capacity of the keramzit cast doubt on the possibility of using the hydroponic method for cultivation of plants on keramzit in space greenhouses.

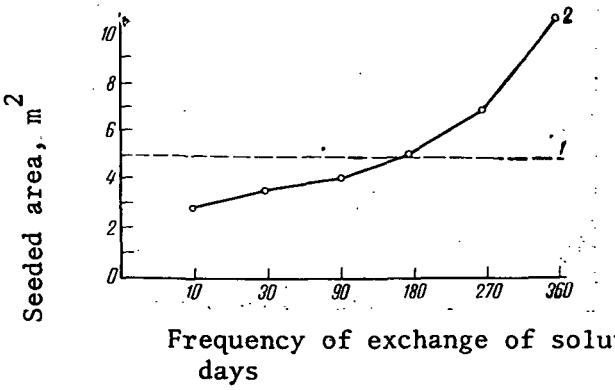

Figure. Change in Area of Greenhouse as a Function of Duration of Use of Nutrient Solution. 1, Calculated area; 2, Actual area.

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CERTAIN INDICES OF THE MATERIAL BALANCE OF MAN AS A COMPONENT IN A CLOSED ECOLOGICAL SYSTEM

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ABSTRACT: In experiments lasting up to six months we show the lack of stabilization in the process of elimination of elements from the human organism when kept on a constant diet. The ratio of minimum and maximum amounts is 1:1.3-2.0. This regularity must be kept in mind when creating, on the basis of products of the vital functions of man, cultural media for autotrophic and heterotrophic organisms which are the separate units of biological-technological life support systems. We discovered the presence of a correlational relationship between certain elements when eliminating them from the organism along with the urine. We showed that this correlational connection is weakened when different stress factors act on the organism.

In order to simulate life support systems in space by recycling of matter /219 a knowledge of the input and output parameters of the determining component of the system, man, is necessary. By input parameters we mean simply the absorbed food, water and oxygen, and by the output parameters we mean the products of the vital functioning of man (urine, feces, sweat, expired air). Establishment of a balanced equilibrium between "input" and "output" is the ideal functioning of the component. We assume that a balanced equilibrium might be achieved by strict correspondence of the diet to the requirements of a man under given conditions and during the absence of neuro-humoral disorders when affected by a given schedule. One of the basic premises for determination of a balanced equilibrium is constancy of body weight. By balanced equilibrium we mean its free and interconnected aspects: the energy and water situations and the balance of the different chemical elements. The parameters which characterize the water balance include the amount of water taken in with food and drink, formed during oxidation of food substances, and removed from the organism along with the urine, feces, sweat and expired air. The parameters which characterize the energy balance are: the values of the respiratory coefficient, the required amount of oxygen, and the energy losses calculated on the basis of the latter; the values for the heats of combustion of the diet, feces and urine and the values for

the exchange computed on the basis of the latter (Smith et al., 1966). Evaluation of these parameters may give an idea of the amount of energy required, assimilated and expended by an organism. The values of exchange energy which are obtained by means of calorimetric analyses and the values for the energy losses which are calculated on the basis of the respiratory coefficient theoretically must agree in maintaining the constancy of body weight.

The parameters which characterize the element balance include values for the consumption and elimination of the different elements by the human organism. Among these are macroelements (nitrogen, sulphur, phosphorus, sodium, etc.) which are detected in the dry residue of daily samples of the ration, feces, urine and perspiration and the microelements (iron, copper, zinc, etc.). The material balance of man in the final analysis determines the elements which are formed in a life support system. In addition, it determines the fluctuation /220
limits of the composition of nutrient solutions and culture media for the autotrophic and heterotrophic organisms which are separate components of the system.

This work is devoted to the study of certain aspects of the material balance of man under varying conditions of diet and schedule. Only the data of chemical-analytical determinations which we obtained in an analysis of the products of human vital activity are discussed. The clinical-physiological problems are not dealt with because the basic purpose of the present studies is an attempt to clarify certain regularities of elimination of elements from an organism. Knowledge of these regularities is necessary for further use of human wastes by autotrophic and heterotrophic organisms and under operational conditions of closed biological-technological systems.

We have summarized the results obtained in seven experiments lasting from one to six months, conducted under conditions of a daily work schedule normal for a scientific researcher and technician under steady-state conditions in a clinic and in a hermetically sealed chamber. The diet used was constant and consisted of standard components. From three to six test subjects participated in each experiment. In all, we examined thirty-eight persons.

One problem which arose during our investigation of the material balance is qualification of the degree of stabilization of the process for elimination of elements from a human organism during prolonged adherence to a strictly constant diet. Because stabilization of the process may be characterized quantitatively by dispersion, we evaluated the significance of differences of dispersions for the values of elimination of elements between the different stages (initial, intermediate and final) of the tests. Our calculations were carried out for a four-month experiment with nourishment from dehydrated products and for a six-month experiment with feeding by the same products inside a hermetically sealed chamber. For explanation of their importance we used the criteria of Fisher (Urbakh, 1964). The values for the Fisher criteria (F), which we found for eight analyzed elements (nitrogen, sulfur, phosphorus, sodium, potassium, calcium, magnesium, chlorine) varied within limits from 1.0 to 2.9 while the critical values of the given criterion which correspond to a 95% reliable level, were 3.2-5.3. Therefore the lack of any shortening of the fluctuation peaks for the values of elimination of elements by the final stage of the tests was mathematically reliable. The fluctuation limits were very great. They stayed the same throughout the entire test. The ratio of the minimum and maximum amounts for urine samples was 1:1.5-2.0; for samples of feces it was 1:1.3-1.5 (Table). It is interesting to note that against a /221
background of such large fluctuations observed for an identical ration and schedule of all the test subjects the individual nature of the process of elimination from the organism could be traced. The latter was confirmed by data of a single factor dispersion analysis during which dispersions due to the factor of individual differences were compared with dispersions due to the factor of random fluctuations. The data obtained in all of the experiments showed that the elimination of calcium is characterized by the greatest individuality and potassium is characterized by the least individuality (F, found for calcium, fluctuated from 7.0 to 28.0; for potassium it fluctuated from 0.2 to 5.6 with critical values of F_{05} from 3.1 to 3.6). The wide fluctuation

limits for a strictly constant diet which were observed in prolonged experiments involving freedom as well as during stationary tests with a control cycle are evidently determined to a significant extent by physiological causes, namely the fact that elimination of elements from an organism depends not only on their overall content in the food but also on the action of other factors (Kaplanskiy, 1938; Genetisinskiy, 1963; Kravchinskiy, 1963). Most important among the latter are the regulatory mechanisms which have a nervous and humoral character (Schottstaidt, 1954).

TABLE. FLUCTUATION LIMITS FOR ELIMINATION OF ELEMENTS IN THE URINE AND FECES DURING A CONSTANT DIET (IN g/DAYS).

Element	Freedom conditions*		Limitation of mobility**	
	Urine	Feces	Urine	Feces
Nitrogen	11.00-17.00	2.10-2.70	13.00-20.00	1.50-2.30
Sulphur	0.50-1.20	0.40-0.60	0.90-1.50	0.20-0.30
Phosphorus	0.70-1.20	0.73-0.98	0.90-1.50	0.50-0.80
Potassium	1.00-2.50	0.55-0.71	2.70-4.60	0.35-0.53
Calcium	0.10-0.20	0.58-0.81	0.15-0.35	0.60-1.30
Magnesium	0.05-0.15	0.30-0.40	0.03-0.10	0.23-0.35
Sodium	4.00-7.00	Traces	4.40-7.70	Traces
Chlorine	5.00-9.00	"	7.60-12.40	"

*Amount of elements in the daily ration (in g): Nitrogen, 20.60; sulphur, 1.55; phosphorus, 2.03; potassium, 3.13; calcium, 0.82; magnesium, 0.53; sodium, 7.10; chlorine, 11.00. **Amount of elements in the daily ration (in g): Nitrogen, 21.00; sulphur, 1.60; phosphorus, 2.00; potassium, 4.00; calcium, 1.20; magnesium, 0.34; sodium, 8.00; chlorine, 12.20.

The problem of the nature of the response of an organism to a change of food factors is important. It was interesting to discover to what degree and how quickly the elimination of elements in the urine reflects a changing level of their consumption with food (the elimination of elements in /222
the feces is determined by the nature of the food itself to a significant degree). In three thirty-day experiments of limited nourishment and in a twenty-day test of complete fasting we found that the elimination of elements,

for which a high percentage of absorbability from food and a clearly pronounced kidney regulation are characteristic, reflects the level of their consumption from the nourishment ration. Among these elements are sodium, potassium, nitrogen, sulphur, and chlorine. The elimination of these elements from the urine decreases with a lowering of the amount of these elements in the food or during a decrease of their absorption by an organism due to a low assimilation capacity. The duration of the transition period for nitrogen, sulphur, and potassium is on the average about seven days; for sodium and chlorine it is about one to two days. One gets a different picture when examining data pertaining to difficultly assimilated deposited elements, whose kidney regulation is weakly pronounced. In this case the level of elimination in the urine may not reflect adsorption of the element with the food. Chief among these elements are calcium and magnesium. The regularity which we mentioned was observed in short-term and in prolonged tests using dehydrated products. In these experiments, as a result of a decrease of the overall amount of calcium in the food or because of its assimilation, the absorption of this element by the organism (calculated for 1 kg of body weight) falls by 1.5 to 2 times in a number of cases. However, the elimination of calcium in the urine remained unchanged during the test periods. The resultant experimental material agrees with the data of many foreign authors, according to whom elimination of deposited elements in the urine is determined by the degree of their saturation in tissues of an organism and does not reflect their consumption in a diet (Malm, 1958; Irving, 1957). The process of adaptation to the corresponding amount of these elements in food may occur for seven months and longer (Mitchell, 1944).

In connection with this account the calculation of the correlational relationship for the elimination of a number of elements to their consumption gives the coefficients of correlation which are below the critical values corresponding to a 95% reliable level. We also observe the high correlational connection between the detected elements during their elimination from the organism in the urine. In the tests each one of the elements detected was correlated by pairs with all the remaining ones. Thus, we calculated the coefficients of correlation for twenty-seven pairs of elements. Calculations were made for each one of the thirty-eight test subjects individually. The

most interesting of the results obtained are data which confirm the existence of a stable positive correlational connection between elimination in the urine for sodium and potassium, potassium and nitrogen, nitrogen and sulphur, potassium and sulphur, and sodium and chlorine. In 70% of the test subjects the value of the coefficients of correlation for sodium and potassium, potassium /223
and sulphur, sodium and chlorine was within the limits 0.7-0.8 with a critical value of $r_{05} = 0.48$. For the element pairs nitrogen-sulphur, potassium-nitrogen the values of the coefficients of correlation were found to be from 0.6 to 0.9 in 100% of the test subjects. A correlational connection between calcium and magnesium with values for coefficients of 0.7-0.8 was observed in only 45% of the persons tested. There was a correlational connection between magnesium and nitrogen and between magnesium and sulphur for an identical number of test subjects. For the element pairs sodium-magnesium, sodium-nitrogen, sodium--sulphur, sodium-phosphorus, potassium-calcium, calcium-magnesium, potassium--phosphorus, magnesium-phosphorus, chlorine-magnesium, chlorine-nitrogen, chlorine-sulphur, chlorine-phosphorus, chlorine-potassium, and chlorine-calcium the coefficients of correlation were not significant. The study of the relationship of elimination of each element to daily diuresis gives significant values for correlation coefficients only for the pairs sodium-diuresis, chlorine-diuresis. The values of the correlation coefficients were 0.8-0.9 in 100% of the test subjects. The dependence of elimination on the value of the daily diuresis was not observed for the remaining elements. We must emphasize that the values for the correlation coefficients were detected during feeding on mixed rations (using dehydrated or natural products) on a daily or stationary schedule. Inclusion of nonstandard food products in the diet (dry biomass of chlorella) or placement of the test subjects in a situation which is abnormal for an organism (hermetically sealed chamber, confinement to bed) cause significant shifts in the nature of excretion of elements in the urine (disturbance of rhythmicity), which produced a weakening of the correlational connection between them. When we did not limit the mobility of any of the test subjects which participated in the experiment, we failed to obtain any correlation coefficient higher than 0.6.

The instability of the process of elimination of elements from an organism kept on a constant diet is expressed in the fluctuation of the value of the material balance when it is determined for a comparatively short interval of time (three to seven days). This value may become negative even with normal conditions for feeding and operating. Deviations of the total three--seven day balance in favor of the positive or negative values was almost 30% of the amount of the element absorbed in the food in a the series of experiments which we conducted. As we mentioned above, one of the necessary premises for establishing a balanced equilibrium is maintaining body weight at a constant rate. According to data in the literature (Smith et al., 1966), a man who weighs 70 kg needs 2,900 kcal/day daily in order to maintain a constant weight under normal conditions; under conditions of a closed space or limited movement he needs 2,300-2,700 kcal/day. In our experiments the calorific value of the /224
assimilated portion of the diets used was estimated on the basis of direct calorimetric analyses of samples of the ration and feces. Then the authors examined the material balance of subjects for whom the calorific value of the ration met the needs of their weight. With the used dehydrated food products having a calorific value of 3,000-3,100 kcal/day during free operating conditions or during comfortable conditions in a hermetically sealed chamber we observed practically no changes in body weight for test subjects having initial weight which did not exceed 75 kg. Nevertheless, its daily fluctuations were 0.1-0.8 kg. Under these conditions experimental determination of balance for a long interval of time (thirty days) showed that establishment of complete balance equilibrium for all elements was not observed. The imbalance was from 1 to 20% of the amount of elements absorbed with the food. It should be pointed out that for healthy persons, 20-27 years of age and under comfortable conditions, there is normally a constant weekly positive balance even when the weight is maintained at one level. In addition, a significant loss of weight by the test subjects was not necessarily accompanied by a negative balance of the important biogenic elements. Thus, for a certain examinees having an initial weight of about 80 kg, weight losses during the experiment were 5-7% of the initial value. However, the balance of nitrogen, sulphur and other elements remained sharply positive throughout all the prolonged tests.

It should be remembered that the elements studied (nitrogen, sulphur, phosphorus, potassium, calcium, magnesium, sodium, chlorine) comprise a negligible portion of body weight (no more than 2-3%), whose fluctuations are determined above all by the balance of carbon, oxygen, and hydrogen.

The results of short-term and prolonged (up to half a year) tests enable us to conclude that there is a lack of stabilization of the process for elimination of elements from an organism during feeding on a constant diet for a period of six months.

By increasing or decreasing the amounts of elements in the foods the level of their excretion from an organism changes, however, the ratio between the minimum and maximum values stays the same (on the average 1:1.2-1.5). This in turn causes an instability of the daily balance of elements.

The instability of the elimination process and the high fluctuation peaks make it difficult to get an accurate adjustment of the nutritive and culture solutions, which are proposed on the basis of the products of vital functions of man, for autotrophic and heterotrophic organisms. In relation to this, it would seem to be efficient to cultivate the organisms in question with a specific range of concentrations of the basic biogenic elements in the medium with preservation of the optimum yields of their production (oxygen, biomass, water, etc.).

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STUDIES OF THE STABILITY OF THE CHEMICAL COMPOSITION OF A CHLORELLA BIOMASS DURING ITS PROLONGED CULTIVATION WITH RECOVERY OF THE MEDIUM ON NITRATES

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ABSTRACT: The chemical composition of a biomass remains sufficiently stable during prolonged cultivation of chlorella with recovery of the medium. Variations in the amount of carbon and nitrogen in the biomass were respectively 1.5 and 5%, and the fluctuations of other basic elements did not exceed 10% of the average value. Fluctuations in the chemical composition were both spontaneous and related to cultivation conditions. Actual fluctuations of the chemical composition of a biomass as a biological feature of the strain used stay within a narrower range than fluctuations which are caused by cultivation conditions that are recorded during the test process.

A necessary condition for inclusion of a biological component in a life-support system is the stability of its basic functional characteristics in time. /225

The metabolism of one-celled chlorella algae was subjected to quite noticeable fluctuations during its cultivation. The metabolic fluctuations which are manifest at this time have both a spontaneous and a dependent nature. In the majority of cases the inconstancy of the metabolic level is related to the recorded changes of the cultivation conditions: the composition of the nutrient medium (Myers, 1951; Krauss, 1958; Tamija, 1955, 1957; Fogg, 1964, et al.), the light intensity and concentration of carbon dioxide (Kylin, 1964; Wintermans, 1958; Sorokin, 1964), the intensity of exchange processes within cells (Semenenko et al., 1966; Iyerusalimskiy, 1966), the pH value (Olsen, 1953; Schaedle, Jacobson, 1965; Scott, 1945, et al.). However, even the stabilization of the external conditions does not prevent fluctuations of the mass-exchange processes in a culture which in this case have a regular, periodic nature and are probably related to peculiarities of the internal rhythm of metabolic regulation within a cell and a population. Gradual changes of the metabolic

characteristics of a culture might also be expected as manifestations of the genetic instability of algae during its prolonged cultivation period.

It is necessary to study all the possible variations of chlorella metabolism under cultivation conditions which are both spontaneous and dependent on possible failures in the stabilization system, in order to make possible the practical use of the component of one-celled algae in a life-support system. /226

This problem has not been treated in the literature.

The direct aim of our work is to study the fluctuation limits of the chemical composition of chlorella cells for long periods of time. This is necessary for quantitative linking of the mass transfer of the algae component with the mass transfer of adjacent components and also for calculation of the supplemental reserves of elements of mineral nutrition for flights of long duration.

In considering this problem it is quite interesting to note not only the data obtained in each separate experiment but also the reproducibility of these data from experiment to experiment over a long period of time.

We conducted five experiments with an overall duration of 164 days. The tests were conducted with the same continuously sustained strain of chlorella Sp-K during 1966-1968 which, with some approximation, could be considered a discrete model of a continuous culture.

Of course this work could be accomplished only after development of methods and apparatus for stabilization of the basic cultivation conditions — the concentration of carbon dioxide and oxygen in the air, lighting, concentration of elements of mineral nutrition and pH, temperature, etc.

In these tests we used a method developed by ourselves for prolonged cultivation of algae with direct recovery of the medium (Meleshko et al., 1967) which was the most economical in terms of weight and energetics and the most likely to be adopted for practical use in a life-support system.

We carried out the cultivation in a rotational type reactor with a closed air circuit with medium recovery on nitrates. The illumination at the surface of the reactor was 35-40 klux.

For the study we used ZN 8 incandescent lamps. The carbon dioxide concentration in the air of the system was maintained within limits of 3-4%, oxygen at 20-30%. Suspension density was stabilized at a level of 10-12 g/ℓ. As the biomass increased the suspension was removed and was centrifuged in 400 mℓ portions, the necessary amount of supplemental solution was added to the centrifuged fluid and a suspension was returned to the reactor through a supplemental solution filling system. We dried the removed biomass to constant weight and analyzed it. The method for adjustment of the medium and the level of maintenance for the separate elements of mineral nutrition in the medium were reported earlier (Lebedeva et al., 1968). /227

We subjected fifty samples of a biomass which had been washed twice with distilled water to an analysis in order to determine the chemical composition.

Data in the form of mean values ($\bar{x}$) with designation of the mean square deviation (σ) and the coefficient of variation (C) in percentages from the mean are presented in Table 1. In addition, similar data for each test are presented in the table.

TABLE 1. COMPOSITION OF THE ELEMENTS IN A CHLORELLA BIOMASS (IN PERCENTAGES OF DRY WEIGHT)

No. of tests	Index	N	P	S	K	Mg
1	$\bar{x}$	8,24	1,01	0,55	1,72	0,28
	σ	0,25	0,05	0,09	0,20	0,04
	C%	3,2	4,9	16	11,6	14,2
2	$\bar{x}$	8,36	1,18	—	1,15	0,42
	σ	0,85	0,08	—	0,03	0,04
	C%	10,1	6,8	—	2,6	9,5
3	$\bar{x}$	8,30	1,43	0,52	1,62	0,55
	σ	0,90	0,05	0,10	0,10	0,16
	C%	10,8	3,5	19,0	6,2	29,2
4	$\bar{x}$	9,35	1,31	0,57	1,04	0,35
	σ	0,32	0,05	0,03	0,10	0,08
	C%	3,4	3,8	5,3	9,6	22,8
5	$\bar{x}$	8,55	1,46	0,45	0,69	0,28
	σ	0,47	0,15	0,06	0,16	0,06
	C%	5,5	10,3	13,3	23,1	21,4
Mean	$\bar{x}$	8,59	1,24	0,53	1,04	0,32
	σ	0,47	0,15	0,05	0,34	0,07
	C%	5,4	12,0	10,0	32,0	22,0

Note: Commas indicate decimal points.

The amount of nitrogen, phosphorus and sulphur in the biomass, whose coefficients of variation were respectively 5.4, 8.0 and 10% turned out to be the most stable. The amount of cations in the biomass fluctuated substantially and the coefficient of variation for potassium was 32, for magnesium it was 22%. We also examined thirty samples of chlorella biomass from various tests for their content of iron whose average value in the cells was 0.14 ± 0.05 and whose coefficient of variation was 35%.

In analyzing the data in Table 1 the difference in the amount of elements in the biomass both in the different tests and throughout each test are of interest. The consumption of nitrogen by the cells from test to test was practically the same with the exception of test 4 where the amount of nitrogen in the biomass was higher and came to 93.5 mg/g as against an average value of 85.9 mg/g of dry weight. At the same time fluctuations in the amount of nitrogen in the biomass within the test limits (tests 2 and 3) were greater than between the separate tests but they did vary in the range of 10% from the average value. /228

The amount of sulphur in the biomass in the different tests was quite stable and its fluctuations from the average value did not exceed 10%. However, during the individual tests (test 3) fluctuations in the amount of sulphur in the biomass were quite significant and the coefficient of variation reached 19%.

Fluctuations in the amount of phosphorus in the biomass were practically the same both in separate tests and between tests while its coefficient of variation was 10-12%.

A large spread was noticed in all of the experiments for the amounts of cations of potassium, magnesium and iron in the algae cells. So the spread in the average values was greater between tests than during individual tests. For potassium the greatest coefficient of variation was observed in test 5 in which it was 23%, while the coefficient of variation for all the tests was 32%.

Usually in the practice of managing a long-term algae culture in industrial installations with low productivity, data of the elementary chemical composition of a biomass are used in calculating standards for introduction of elements.

However, the data in Table 1 cannot be used as reference data for compiling the supplemental solutions for the purpose of stabilization of the mineral elements in the medium because elements are carried away from the medium not only by cells but also with the medium as well as with microflora. At the same time even the slightest discrepancy between a loss and replenishment of elements very quickly leads to a decrease or a buildup in the medium of one element or another during the prolonged intensive cultivation of chlorella with recovery of the medium. With this in mind we examined the loss of mineral elements along with unwashed biomass in all of the tests.

Table 2 presents data of a study of the composition of samples of washed and unwashed biomass taken in parallel in four tests.

TABLE 2. COMPOSITION OF ELEMENTS IN A CHLORELLA BIOMASS (IN % OF DRY WEIGHT).

Biomass	Index	N	P	S	K	Mg	Fe	Ashes
Unwashed	$\bar{x}$	8.60	1.61	0.50	1.09	0.48	0.28	9.3
	σ	0.12	0.11	0.06	0.12	0.05	0.08	1.6
	C%	0.15	6.85	12.0	11.0	10.4	28.0	17.0
Washed	$\bar{x}$	8.55	1.46	0.45	0.69	0.32	0.15	6.1
	σ	0.17	0.15	0.06	0.19	0.05	0.03	0.9
	C%	0.2	10.3	13.0	23.0	17.0	20.0	15.0
% of loss in the unwashed	99.5		90.7	90.0	63.4	62.5	53.5	65.5

From these data it is evident that the absolute loss of mineral elements along with the unwashed biomass is greater than with the washed. However, this difference for the various elements fluctuates significantly. Thus, for nitrogen it is statistically unimportant and for phosphurs and sulphur it is reliable but not very great in volume (10%). The loss of cations (K, Mg and Fe) with the cells is respectively only 63, 62 and 54% of the general loss with the unwashed biomass.

Data which were obtained in a prolonged cultivation of chlorella with recovery of medium with different variations in cultivation conditions that are actually met in prolonged tests are interesting for their practical use in a system for stabilization of medium composition. For this purpose we used material from experiments with an overall length of 164 days for which 70 samples of unwashed biomass were analyzed (Table 3). /229

TABLE 3. COMPOSITION OF ELEMENTS IN UNWASHED BIOMASS OF CHLORELLA (IN PERCENT OF DRY WEIGHT).*

Index	N	P	S	K	Mg
$\bar{x}$	8,57	1,58	0,52	1,19	0,55
σ	0,35	0,13	0,04	0,18	0,08
C%	4,0	8,2	7,7	15,1	14,0

*Translator's note: Commas indicate decimal points.

The table presents average values $(\overline{x})$ with indication of the mean square deviation (σ) and the coefficient of variation (C) in percentages of the mean value.

These data were taken as reference for compilation of the adjusting solution and for calculation of the reserve of salts during prolonged cultivation of chlorella with medium recovery.

From the data in the table it is evident that the amount of nitrogen, sulphur and phosphorus in the unwashed biomass fluctuates within the same limits as it does for the washed samples. The fluctuations are less for the cations and the coefficient of variation for potassium is 15.1% and 14.0% for magnesium.

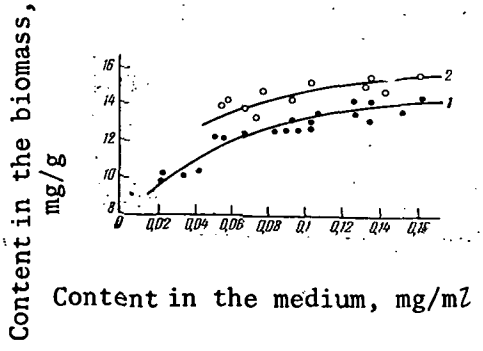

Figure 1. Amount of Phosphorus in the Washed (1) and the Unwashed (2) Biomass of Chlorella in Relation to the Concentration of Phosphorus in the Medium.

In the designated tests we also determined that the average value of the /230
loss of nitrogen, sulphur, potassium and magnesium by the cells did not depend on their concentration in the medium. The buildup of phosphorus in the biomass depended to a significant degree on its concentration in the medium. Therefore it is not possible to evaluate its repetition of buildup without consideration of the composition of the medium in which the biomass is cultivated. In this regard we compiled data obtained in four tests (except test 1, Table 1) of the amount of phosphorus in the composition of a biomass and the concentration of inorganic phosphorus in a medium in which it was cultivated.

In Figure 1 we show the relationship between these values for the unwashed and washed biomasses. As is evident from the drawing, by changing the concentration of phosphorus in the medium within the limits 0.11-0.03 mg/mℓ, the buildup of this element in the chlorella cells was 13 ± 0.7 mg/g, and the mean square deviation did not exceed 5% of the average value. After lowering the background concentration of phosphorus its losses decreased noticeably and with a concentration of 0.02 mg/mℓ they reached 10 mg/g of dry weight.

The greatest fluctuations in the cells' consumption of mineral nutrition in great part were chiefly due to the recorded disturbance of the cultivation cycle. For example, when the light was accidentally turned off in test 2 and the concentration of carbon dioxide increased we noticed a loss of phosphorus, sulphur, potassium and magnesium from the cells which was accompanied by an increase of the concentration of these elements in the medium. In test 5 the consumption of elements of mineral nutrition by the cells varied in connection with an accidental fall of the pH of the suspension to 3.0.

Below we will present the data of the basic indices of metabolism for chlorella in test 5 in which there was an accidental lowering of the pH on the twenty-first day.

Figure 2 shows the losses of elements in the biomass in this test. One's attention is drawn to the existence of at least two basic levels of losses for the majority of the elements. This peculiarity is most pronounced for phosphorus whose consumption in the first stage was from 18 to 20 mg/g of dry weight, and during the second stage from 14-17 mg/g although the concentration of phosphorus /231

in the medium in the first half of the test was only slightly higher than during the second. It was difficult to explain such an unusually high consumption of phosphorus which we had not previously observed. We decided to conduct a fractionation of the phosphorus in the biomass in order to determine which fraction, organic or mineral, caused this increase to occur.

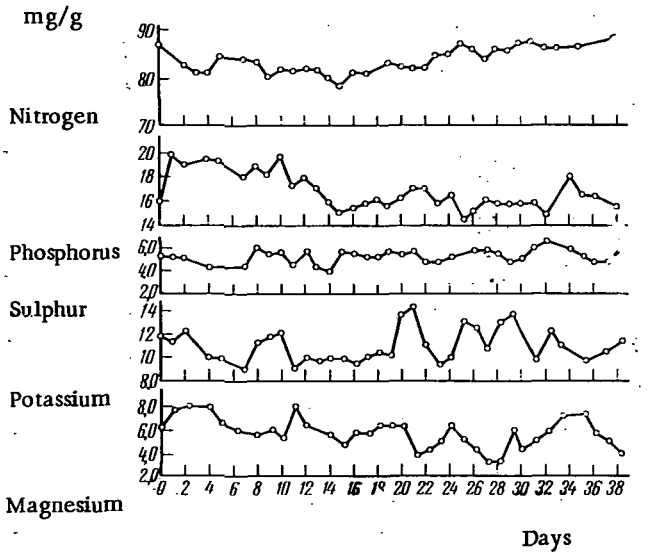

Figure 2. Amount of Elements in the Chlorella Biomass During Its Prolonged Cultivation on a Nitrate Medium. (Test 5).

The data in Table 4 show that the increase in phosphorus in the biomass was related mainly to the fraction of organically bound phosphorus which is an indication of the intensification of exchange processes within the cells of the chlorella.

Sometime after the stabilization of phosphorus at the lower level (on the 22nd day) the second level for nitrogen (84-87 mg/g) showed up clearly, and in contradiction to the phosphorus it was higher than the first (81--84 mg/g). The total loss of anions of NO_3^-, $H_2PO_4^-$, SO_4^{-2} at both levels remained practically unchanged and was 6.85 mg · equiv/g of dry weight.

The tendency of changes in the amount of potassium and magnesium in the biomass was directly contradictory: an increase in the amount of potassium in the biomass during the second period corresponded to a reduction in the amount of magnesium while the absolute fluctuations of each of them were comparatively high. If we compare the average values for each of the periods, then in the second period the total of the cations ($K^+ + Mg^{+2}$) reduced to 0.70 in comparison with 0.81 mg × equivalent/g in the first period.

TABLE 4. AMOUNT OF ORGANIC AND MINERAL PHOSPHORUS IN THE BIOMASS (IN mg/g OF DRY WEIGHT).

Days from beginning of the test	Phosphorus in Fractions		
	Total	Mine	Organic
5	19.3	6.2	13.3
8	19.0	6.6	12.4
10	19.5	6.8	13.7
Average	19.3	6.2	13.1
23	16.8	5.2	11.6
30	15.7	6.1	9.7
38	15.2	5.3	9.9
Average	15.9	5.5	10.4

A complete analysis of the biomass including the amount of ash element and /232
also the amount of carbon, nitrogen, oxygen and hydrogen enabled us to calculate the degree of reduction of carbon in the cells (R) and find the empirical formula for the organic matter of the chlorella cells at both stages of cultivation (Table 5).

TABLE 5. COMPOSITION OF CHLORELLA BIOMASS BY PERCENT OF DRY WEIGHT (TEST 5, AVERAGE DATA FROM SIX DETERMINATIONS).

Days from beginning of test	C	H	N	O	Ash elements	Assimilation coefficient of CO_2/O_2	R	Composition of organic matter
1-20	47.16	6.94	8.3	32.60	5.0	0.83-0.85	39.1	$C_{6.6}H_{11.7}O_{3.4}$
21-38	51.28	6.95	8.5	28.27	5.0	0.75	43.2	$C_{7.0}H_{11.4}O_{2.9}$

The data in Table 5 shows that reduction of the organic matter in the first half of the test was lower (39.1) and corresponding to it there was a higher value of the assimilation coefficient (0.83), but during the second half of the test, on the contrary, reduction of the organic matter became higher and the assimilation coefficient correspondingly lower (0.75). In an analysis of the data of the dynamics of the assimilation coefficient in the test we pointed out /233 that the assimilation coefficient and consumption of carbon and hydrogen also revealed two levels. The assimilation coefficient decreased in the second half of the test from 0.83 to 0.75 and simultaneously the amount of carbon in the cells increased from 46-48 to 50-52%.

Thus, an analysis of the data showed that the intensity and orientation of the exchange processes in the cells of chlorella did not remain the same throughout the test. It is true that the absolute quantitative difference is not very great but it did show a clear qualitative distinction.

In relation to what has been written it is natural to call one's attention to the structure of the chlorella population which was accumulated during the test. During cultivation of algae under continuous lighting an asynchronous culture is always formed for which individual species are found at different stages of the ontogenetic development. At the beginning of the experiment the steady dynamic equilibrium between a number of species of different physiological age may shift to one side or the other during the change in cultivation conditions.

In the experiment described it turned out that the culture also had two levels in relation to the cells of different physiological age. During the first twenty days of cultivation the photosynthetically active growing cells predominated while during the second period the relative number of morphogenetically active cells increased sharply (autospores and mother cells) (Table 6).

From Table 6 it is evident that the number of photosynthetically active species in a population in the second half of the test was almost two times less than in the first half. However, the decrease of physiologically active species in the population did not reflect on the amount of productivity of the

culture which decreased only by eight to ten hours after overoxidation of the suspension but remained stable the remainder of the time.

TABLE 6. STRUCTURE OF THE POPULATION IN TEST 5. (AVERAGE DATA IN PERCENT OF THE OVERALL NUMBER OF CELLS).

Days from beginning of test	Photosynthetically active cells	Mother cells	Autospores
1 - 20th	60 ± 7.0	26 ± 6.0	14 ± 6.0
21 - 38th	37 ± 8.0	36 ± 8.0	28 ± 8.0

Above we spoke about the connection of the pronounced change in the level of consumption of elements of mineral nutrition with the sharp increase in /234 acidity of the suspension for 1-2 hours. However, we cannot consider this circumstance the true cause for a change in the level of exchange which was maintained for the following seventeen days of the test or approximately during twenty generations of cells. It would be more correct to assume that this short-term disturbance of cultivation conditions is only a triggering action which unlocks the internal mechanisms for adjustment of metabolism. In this given instance, evidently we must speak about genetic changes insofar as the effect was observed for many generations. From this we can conclude that the spread in amount of elements in the biomass between different tests which were conducted over a period of three years might be due to different preparation conditions of sowing material (rising of the culture to a working density, centrifuging conditions, etc.), and also to several varied conditions which occur during the experiment (amount and quality of metabolites, qualitative and quantitative composition of microflora).

Considering this unstable exchange, we may conclude that "spontaneous" difference in the absorption of elements in different tests may be explained by the distinctive prehistory of the culture. Actual fluctuations in the chemical composition of the biomass such as the biological characteristics of the strain which we used, keep within narrower limits than fluctuations caused by conditions of cultivation which are recorded in the process of the tests.

In designing a component of one-celled algae, one must keep in mind the possibility of the changes of metabolism of the cells which do not have a visible connection with cultivation conditions. Such changes may be determined by the physiological and cultural quality variation of the initial sowing material or observed in actual conditions during replacement of a culture for any reasons by a new one from the reserve supply which is stored in the storehouse on the ship.

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USE OF PRODUCTS OF BIOLOGICAL MINERALIZATION FOR CULTIVATION OF HIGHER AND LOWER AUTOTROPHS.

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ABSTRACT: This work reports the data of the use of products of the vital functions of man which are mineralized by a biological method.

On the basis of these products nutrient solutions were made and subjected to biological tests using lime and higher autotrophs.

The results of the study showed that the productiveness of the test plan did not differ from the control variants. that is, the nutrient mixtures did not contain toxic substances, which means that they could be used with adjustment for the elements they are deficient in for root feeding for cultivating higher and lower plants.

The close interrelationship and mutual influence of the separate elements /23
in a biological-technical life support system impose rigid methodological requirements for the study and development of these elements which are not isolated from one another and require consideration of the input and output parameters of the adjoining units and components.

In particular, development of the unit for biolcgical mineralization of the products of the vital functioning of man must, above all, solve the problem of the suitability of products of mineralization as a basis for a nutrient medium of a component of autotrophic organisms which follows it. In the waste products of the vital activity of man there are physiologically active substances, traces of which may remain in solution after biological mineralization and have a stimulating or inhibiting effect on the growth and development of autotrophic organisms. The problem of the specificity of the action of nutritive media, which are formed on a basis of urino-fecal liquids, has not been studied and is of undoubted interest. Therefore, the aim of our tests was to determine the possibilities for cultivation of higher and lower autotrophs on nutrient media which include the products of the vital activity of man after biological mineralization. It has been supposed that by means of

cultivation of lower and higher plants (biological control) it would be possible to evaluate the specificity of the influence and degree of correspondence of the chemical composition of the final products of mineralization to the requirement of the autotrophs.

Presented in this report are the results of studies of the use of nutrient /236
solutions which contain products obtained in different technological arrangements and modes of operation of a unit for biological mineralization. In the biological mineralization unit we used the aerobic process achieved by a polyculture of microbes-mineralizers (active sediment). A continuous flow system was used for cultivation of microorganisms, in one case with recirculation of the culture on a unit of the "aerotank-regenerator" type, and in another in chemostat conditions on the "ANKUM" unit (apparatus for continuous cultivation of microorganisms). The ratio of solid and liquid products of the vital activity of man was always constant: one part fecal and two parts urine. The concentration of the starting mixtures was varied.

When we were carrying out the tests the degree of mineralization of the starting mixtures also varied in a wide range which was achieved by a change in the technology of the process. Thus, for tests with higher plants the composition of the mineralized solutions which we obtained from the "aerotank--regenerator" type of unit fluctuated within the limits: COM (Chemical Oxygen Minimum) 200-400 mgO_2/l; $N_{NH}4$ — 94-210 mg/l; N_{NO_2} — 10-35 mg/l; N_{NO_3} — 10-82 mg/l; P — 20-32 mg/l; K — 48-73 mg/l; Cl — 270-440 mg/l; Na — 66-137 mg/l.

For tests with lower plants the composition of the mineralized solutions from the "ANKUM-1" unit on the average was the following: COM — 1,680 mgO_2/l; N_{NH_4} — 680 mg/l; N_{NO_2} — 1.3 mg/l; N_{NO_3} — 0; P — 23 mg/l; K — 260 mg/l; Cl — 569 mg/l; Na — 195 mg/l. One group of higher plants was cultivated on a Knop solution, the other was cultivated on a nutrient solution which included products of biological mineralization.

Products of biological mineralization were used as an adjusting solution which entered the "biological test unit" daily. The "biological test unit"

for higher plants was a luminostat with an area of 1 m^2, equipped with twenty fluorescent lamps, a time relay, a fan, thermographs and psychrometers. Underneath the lamps we distributed four containers (with an area of 0.25 m^2 each) for cultivation of plants.

The object of the investigation was Chinese cabbage with a planting density from one to twenty-four days — 120 plants/m^2, from twenty-five to forty days — sixty pieces/m^2. The duration of the light period was eighteen hours. The intensity of the illumination was 2,000-12,000 lux/m^2. The method of growing was a water culture having a nutrient solution volume of twenty liters on an area of 0.25 m^2. The test lasted forty days. The test was repeated twice.

The solution in the amount of 1.5 ℓ obtained from the biological mineralization unit was poured into the nutrient solution once every three days. Then /237
the medium was added to to make a concentration equal to the level of the Knop solution.

We maintained control of the nutrient solution regarding its concentration of basic elements of mineral feeding. The pH of the solution was adjusted daily to 5.6-5.8. The consumption of water was figured once every three days. The criterion for suitability of the mineralized solution was the productivity of the plants based on dry and moist matter in comparison with plants grown on a nutritive Knop solution. The results of the test showed that the productivity of plants which had been grown on a mineralized solution did not differ from that of plants which had been grown in the Knop solution (Table 1).

TABLE 1. YIELD OF BIOMASS (IN GRAMS) FROM AN AREA OF 0.25 m^2 FOR FORTY DAYS.

Biomass	Moist weight		Dry weight	
	Control	Test	Control	Test
Leaves	1,361.2	1,381.1	64.92	67.46
Roots	110.0	101.1	8.83	7.13
Total wt.	1,471.2	1,482.2	73.75	74.59

Throughout the test we periodically made corrections of the nutrient solution: the test variant was corrected with products of mineralization and by salts; the control variant was adjusted with salts.

Both variants were filled up to the level of concentration of the nutrient Knop mixture. During the test (forty days) we administered N – 5,330 mg, P – 1,380 mg, K – 2,750 mg. A rather significant part were elements which had been introduced together with the mineralized product (in mg):

	N	P	K
Total amount.	5,531	1,383	2,750
Administered with mineralized products	3,368	430	1,046
Correction for the mineralized products, %	61.0	30.1	38.0

Absorption of the elements was determined by the difference between the starting and residual contents in the nutrient solution

As can be seen from the data in Table 2, the absorption of the basic elements of mineral nutrition and water in the control and in the test turned out the same.

As we indicated above the urino-fecal mixtures were subjected to biological control using lower plants. These mixtures had been mineralized on the "ANKUM" unit. The resultant solutions were adjusted by mineral salts to the values of the Tamiya medium. The object of the study was the thermophilic strain of chlorella Sp-K.

TABLE 2. ABSORPTION OF N, P, K (IN mg) AND H_2O (IN ℓ) FROM THE NUTRIENT SOLUTION.

Days of test	N		P		K		H_2O	
	Control	Test	Control	Test	Control	Test	Control	Test
From 1st to 10th day	528	684	184	202	457	670	3.0	3.07
From 11th to 20th day	637	708	152	185	951	986	7.50	7.03
From 21st to 30th day	1,909	1,848	497	416	2,011	1,600	12.80	11.55
From 31st to 40th day	2,245	2,560	629	412	2,190	2,239	16.45	16.20
Total	5,329	5,800	1,462	1,215	5,609	5,495	39.75	37.85

Cultivation of chlorella in adjusted solutions was carried out in Erlen- /238
meyer flasks with a suspension volume of 150 ml. In order to detect the presence in the medium of substances which are toxic for the growth of chlorella, its initial concentration in the enrivonment was varied (7 million parts, 65 million parts and 155 million parts/kl/ml). For the control, the chlorella was cultivated in a Tamiya medium. The algae was cultivated in a "Lyuminostat" unit with illumination of 10,000 lux by TBS-30 luminescent lamsp. A mixture of air with 1-3% carbon dioxide was bubbled through the culture. The bubbling rate of the suspension was 2 l/min. Temperature was maintained at a level of 37-38°C. Duration of the tests was four days.

During the running of the experiments, the following parameters were determined daily: the concentration of chlorella cells (photometrically), the pH of the solution and the morphological condition of the cells. The criterion of suitability of the mineralized solution was the growth of the biomass of chlorella in comparison to the growth of the biomass in similar conditions in a Tamiya nutrient solution.

Results of the experiments of cultivation of chlorella in solutions obtained after biological mineralization of the aqueous-urinal-fecal mixtures which are presented in the diagram show that the growth of chlorella in mineralized solutions is not less than the growth of the chlorella in the controlled Tamiya medium.

In addition, we noticed no morphological deviations from the normal in chlorella. The normal growth of the chlorella in the test medium with a low starting concentration of chlorella (7 million kl/ml) confirms the presence of toxic substances in the mineralized solutions which inhibit its growth.

From all of the above, we can make the following conclusions:

Inclusion of products of the vital functioning of man into the adjusted solution after biological mineralization show the possibility of its use for /239
cultivating higher plants by adding the missing elements;

The urino-fecal mixture which is mineralized by the biological method is fully suitable for cultivation of chlorella after appropriate adjustment.

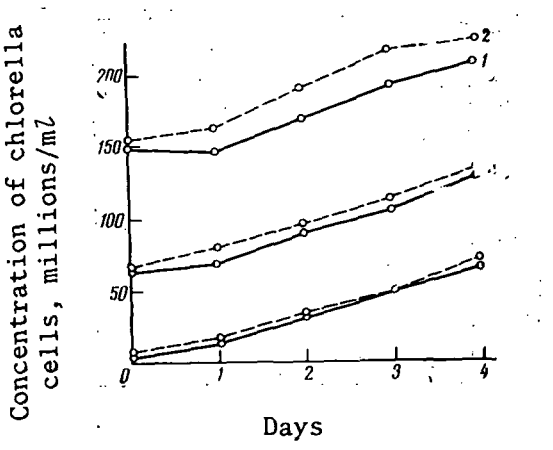

Figure. Growth of Chlorella in Urino-Fecal Mixtures Mineralized by the Biological Method. 1, In a Tamiya medium; 2, In mineralized media.

PROBLEM OF THE POSSIBILITY OF USING PRODUCTS OF THE VITAL FUNCTIONING OF MAN WHICH HAVE BEEN MINERALIZED BY THE "WET BURNING" METHOD

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ABSTRACT: This article presents experimental data on the raising of higher plants under artificial conditions by the hydroponic method on a porous clay filter ("keramzit") using fecal masses which have been mineralized by the "wet burning" method in the composition of the adjustment solution.

In the course of a year during the experiment, no reduction in the productivity of plants in dry and wet matter was noticed.

The incompletely oxidized organic compounds of the mineralized products were not incorporated in the nutrient solution during the experiment but were absorbed by the substrate or completely mineralized by biological means and had no toxic effect on the plants.

In creating a closed-cycle system, the products of the vital functions of /239
man and biocomplex must be mineralized and returned to the system. Their mineralization may be accomplished by physical-chemical or biological means.

The studies which we carried out had the purpose of examining the possibility of the use of products of the vital functions of man mineralized by "wet burning" for the nutrition of higher plants and also to evaluate the specificity of the action of these products in relation to the duration of their use.

"Wet burning" is one of the methods of physical-chemical mineralization in which the oxidation of organic substances is carried out in a solution with /240
increased temperature and increased pressure. The mineralization of organic substances in a liquid medium differs from mineralization by other physical-chemical methods not only by conditions of the oxidation process but by the fact that after mineralization, the substances are found mainly in the dissolved and partially in the dispersed state.

Human feces were mineralized by "wet burning" in our studies. The mineralization was carried out in rotating autoclaves with periodic action for

one hour at a temperature of 275° and pressure of 150 atm. The degree of mineralization of the organic substances reached 80-85%.

The solutions obtained after mineralization of the aqueous-fecal mixture (30 grams of feces to one liter of water) have the following composition. The average chemical composition of the mineralized solution (in mg/ℓ) is given below:

Nitrogen		phosphorus	27-60
Ammonia	188-468	potassium	65-120
Nitrate	0-2.6	sodium	15-32
Nitrite	0-0.3	chlorine	210-238

In a closed system products of the vital functions of man are formed daily. If we decide that a definite area of plant growth is to be set aside to provide man with food then it is evident that the mineralized products of the vital functions of man may be used only on this area. Based on the given ration we computed that about 20 g of feces a day will be necessary for one m^2 of sowing area. This calculation was accepted during the test but because we had to make adjustment of the nutritive solution once every three days, we had to add 60 grams of mineralized feces to the adjustment solution.

The test was carried out in open units on keramzit with periodic watering with a nutrient solution. Plants which were cultivated in solutions containing mineralized salt were the controls. The test was conducted for a year and consisted of four independent plantings of vegetable crops which were similar in their requirements for growing conditions and which were included in the planting corresponding to their share in the given human ration.

Before the beginning of the test we compiled a standard solution from mineralized salts, based on the calculation of 80 ℓ to 1 m^2 of planted area for both variants. The adjustment solution for the first variant was composed of mineral salts and for the second variant it was composed of products of the mineralization of feces with addition of the missing elements. Throughout the /241 tests both adjusting solutions were balanced in their contents of basic

elements of mineral nutrition, and the concentration of these elements changed in relation to the plant's requirement of them at different growth periods.

The vegetational period of one planting in the test was 90 days. At the end of this period, the plants were gathered and analyzed and new plants were planted in their place. The nutrient solution and the substrate were not changed throughout the test. Keramzit with a 5-15 mm fraction, with a root--inhabited layer of 18-20 cm was selected as the substrate.

As we have said above, the collection of vegetable crops was planted in a definite combination in the test. Under the conditions used in the test (common planting area, common nutrient solution, high plant density, etc.), the plants undoubtedly had a stimulating or depressing influence on each other's development (Chernobrivenko, 1956; Tokin, 1948; Grodzinskiy, 1965; Gryummer, 1956, et al.,). Therefore, in our opinion, it is not advantageous to consider the results of the influence of the products of mineralization on productivity of separate crops in mixed planting, but it is more correct to view the productivity of planting as a whole.

The data in Figures 1 and 2 obviously confirm the fact that inclusion of fecal masses mineralized by "wet combustion" into the adjusting solution was not reflected in the productivity of the plantings of dry and moist matter. Certain deviations which were observed in the third planting did not show the effect on the yield of plant production for a year. Therefore, for the year the weight of moist biomass in the control was 86.9 but in the test variant it was 85.2 kg/m^2. Of these amounts there were respectively, 54.9 and 54.5 kg/ /m^2 of economically useful part of the yield. Similar data were obtained for the yield of dry matter for the year.

The reduction of the productivity of plants as a function of the duration of the test was parallel in both variants and was caused by other factors (salinization and aging of the substrate, accumulation of ballast ions in the solution, etc.).

We determined the consumption of elements of mineral nutrition from the nutrient solution according to the results of analyses conducted periodically.

The data obtained by this means give the total consumption of these elements for feeding of the plant and accompanying microflora and also for the absorption of the substrate.

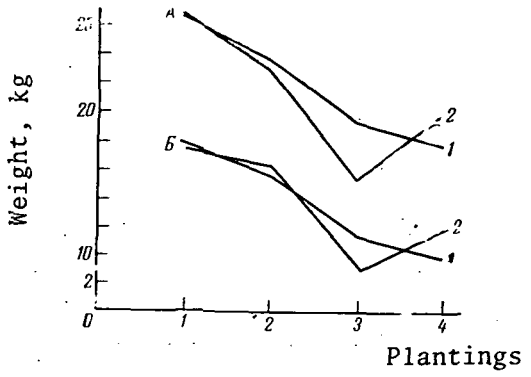

Figure 1. Effect of Mineralized Solutions on Productivity of Plants of Moist Material (in kg/m^2) in Relation to Duration of Application. A, Overall weight of biomass of the plants; B, Weight of the economically useful part; 1, Control; 2, Test.

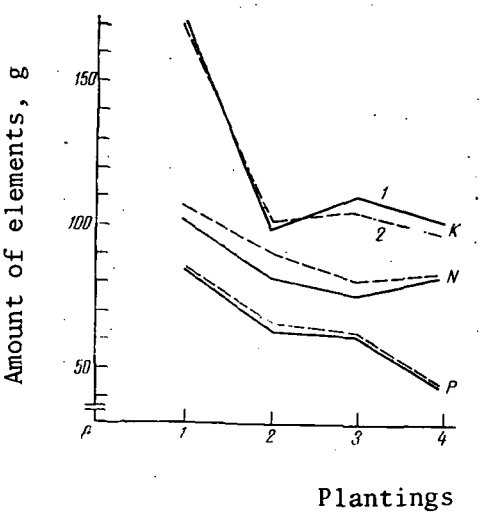

Figure 2. Effect of the Duration of Application of Mineralized Solutions on Productivity of Plants for 90 days (in kg of dry substance to 1 m^2). Designations are the same as Figure 1.

As can be seen in Figure 3, the absorption of the basic elements of mineral nutrition is very close in both variants for all of the plantings in the test. The total consumption of nitrogen for the year was 336 g in the control and 358 g in test variants, 249 g and 253 g, respectively, for phosphorus, and 478 and 468 g, respectively, for potassium.

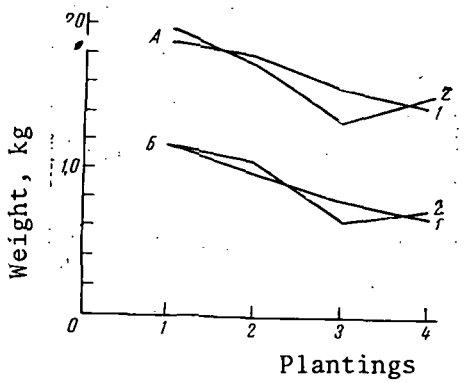

Figure 3. Absorption of N, P, K During Vegetation (in g for 1 m^2 of Planting). Designations are the same as for Figure 1.

The decrease of absorption of N, P, and K in the test period was identical /242
for the two test variants and consequently, could not have been caused by action of the mineralized products.

The greatest demand for elements of mineral nutrition occurred during the first planting. This was caused by the great adsorptive capacity of the substrate (Revut, 1965). A number of our studies determined that in the ninety days the keramzit was able to absorb up to 72% of the phosphorus, 68% of the nitrogen and 69% of the potassium of the solution without reaching saturation.

In subsequent plantings the absorption of nitrogen and potassium somewhat stabilized while the absorption of phosphorus continued to fall. In our opinion, the cause of this was the large buildup of phosphorus by the substrate. Thus, analysis of a hydrochloric acid extract (0.2 N, HCl) from the keramzit showed that the reserve of phosphorus in the substrate which reached a plant by the end of the test had increased by more than 400 times and reached 247 mg of P_2O_5 /243 to 100 g of keramzit. Such a high buildup of phosphorus in the substrate might be one of the causes for reduction of the plant yield. After all, soils which contain 25 mg of P_2O_5 to 100 g are considered well-supplied with phosphorus.

The ratios of elements absorbed per year (N : P : K) are close in both variants and comprise 1:0.74 : 1.31 in the control and 1:0.70 : 1.42 in the test variant, although in separate plantings more significant shifts were noticed.

The growing of plants on products of vital functions which have been mineralized by "wet combustion" method was also not reflected in the consumption of water. For example, during a year, 1,692 l of water were lost to transpiration and evaporation from the surface of the substrate in the control and 1,730 l in the test variant.

As we have already mentioned above, the solutions which were obtained after mineralization of the fecal masses by the "wet combustion" method contained a quite significant number of organic compounds. The chemical oxygen minimum (COM) of these solutions in our test fluctuated from 1,400 to 2,400 mg/l of O_2 (bichromate oxidizability).

In view of the fact that the mineralized solution in the test was applied periodically and in comparatively small portions (2 l of mineralized solution to 80/l of standard), it was wondered if the residual supporting structure would begin to accumulate during prolonged administration of these solutions and what its effect would be when the ratio of the administered mineralized solution to the standard becomes 1:1, 1:2, etc. Therefore, one of the tasks of the investigation was determination of the threshold concentrations of the organic compounds in order to simultaneously provide regeneration of the nutrient solution.

During the experiment, we introduced 202 l of mineralized solution to 1 m^2 of planting area (i.e., in a ratio of it to the standard of 1:2.5), which corresponded to an administration of 6.06 kg of feces. With this solution, there /244 was an amount of supporting structure, which showed up in 389,000 mg of O_2 according to the COM. The oxidizability of the nutrient solution after the year-long experiment was 7,700 mg of O_2, i.e., the supporting structure in the amount of 381,000 mg of O_2 was mineralized or absorbed by the keramzit and did not have a toxic effect on the productivity of the plant.

If this had not occurred, then the COM of the nutrient solution at the end of the test would have been about 5,000 mg of O_2 to 1 l, but naturally, this solution was not suitable for the cultivation of plants. The process of mineralization, or absorption of supporting structure, proceeded rather quickly, Thus, the COM of the nutrient solution was 80 mg/l before adjustment, and after adjustment it increased to 128. However, after only one day the COM of this solution decreased to 90 mg of O_2.

Absorption of the supporting structure of the nutrient solution on the surface of the keramzit is also confirmed by data of infrared spectrometry. Spectra of extracts of the keramzit by carbon tetrachloride before and after cultivation of the plants are shown in Figure 4. As can be seen in the spectra shown, principally high molecular hydrocarbons are detected

in the extract before cultivation of the plant (length of chain C_{19}[1]), when after CCl_4 cultivation substances are extracted which might develop into fatty acids of their complex esters (chain length C_{13}[1]).

Loss of supporting structure from the nutrient solution (Figure 5) occurred uniformly during the entire experiment: during the first planting it was 91; in the second, it was 89; in the third, 94, and in the fourth, 92%.

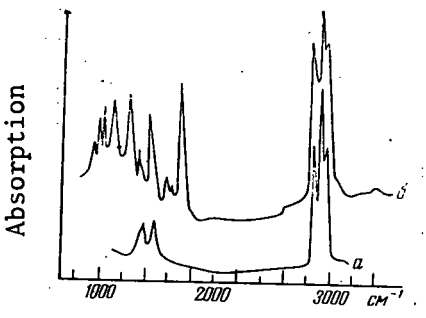

Figure 4. Spectra of Extracts of Keramzit CCl_4 (Cuvette of NaCl, d = 0.27). a, Before cultivation of plants; b, After cultivation.

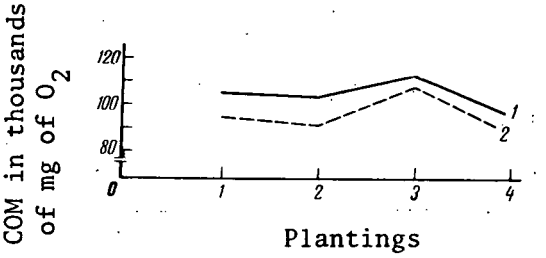

Figure 5. Amount of Supporting Structure and Its Loss From the Nutrient Solution During the Test Periods. 1, Administered supporting structure; 2, Absorbed supporting structure.

This circumstance indicates that a biological mineralization of the supporting structure by accompanying microflora occurs in addition to the absorption by the substrate. This microflora actively colonizes the keramzit in the hydroponic installations and, according to the data of Ye. N. Yermakov (1965) may not be less than the microflora of a peat mixture in type and in quantity of composition.

The high mineralizing capacity of microflora which colonizes the keramzit is also evident by its use in biofilters for purification of waste waters (Postnikov, Kharitonov et al., 1963).

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[1]The chain length was estimated by the relationship $D_{\nu CH_2}/D_{\nu CH_3}$.

All of the above enable us to conclude that: /245

In cultivation of plants on keramzit, the prolonged use of fecal masses mineralized according to the "wet combustion" method in a composition of the adjusting solutions is possible without reducing their productivity in comparison with a culture on a solution of mineral salts;

During prolonged use of fecal masses mineralized by the "wet combustion" method, the residual supporting structure does not accumulate in the nutrient solution but is absorbed on the surface of the substrate and evidently is biologically mineralized.

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PROBLEM OF MINERALIZATION OF VEGETATIVE WASTE PRODUCTS OF A BIOCOMPLEX BY THE METHOD OF THERMAL COMBUSTION

I. V. Tsvetkova, B. G. Gusarov, V. P. Zamota, Ye. V. Maksimova and L. A. Filatkina

ABSTRACT: Carrying out the process of mineralization of waste products depends to a great extent on the specific peculiarities of the culture and the conditions of their cultivation. During cultivation of plants on keramzit, (aluminoferrosilicate) heavy metals (Al, Fe, Mn, Zn) are washed away due to the effect of root discharges and they accumulate in the root systems. The buildup of elements with changing valences evidently may have a catalytic effect during the use of the thermal combustion method. The greatest quantity of metals is built up in the economically nonuseful part of cabbage which brings about damage to the oxide film of the working surfaces of the burners. Because these plants may become part of the human ration, it is necessary to provide for a number of requirements for the earth burner devices in order to carry out the method of thermal mineralization.

The creation of biological-technological life-support systems which are /245
based on the cycling of substances is impossible without using the products of mineralization of the vital functions of man and the waste products of the biocomplex. Mineralization may be accomplished on a base of physical-chemical and biological methods. Among the physical-chemical methods of mineralization, the method of high-temperature burning (700-900°) in the presence of an oxidant may be used. Realization of the mineralization process under the given conditions makes necessary the use of special heat resistant materials in the /246
reactor. We used oxygen in the air as an oxidant in the test with a consumption rate of four nm^3/hr. Vegetative waste products, that is, the economically nonuseful part of certain agricultural plants, were mineralized under different operating conditions by the method of thermal combustion. The data obtained showed that in the case of combustion of radish, carrot and beet greens, the material of which the burner devices are made satisfies the heat requirements. However, there is damage to the working surfaces of the burner

devices during mineralization of the waste products of Chinese and white-headed cabbages. The damage was noticed after mineralization of only 100 g of dry biomass of cabbage waste products at a combustion temperature of 700°.

We might have supposed that damage to the working surfaces of the burner devices during mineralization of the vegetable waste products from Chinese and white-headed cabbages was determined by the action of some substances or elements which accumulate in these plants in amounts which are capable of causing metal corrosion. The action of cabbage waste products on burner devices might be related to certain specific peculiarities of these plants which were manifested in concrete conditions of cultivation. In our investigation we attempted to explain these hypotheses. The plants of the biocomplex were cultivated in open devices with a planting area of 1 m^2 and with artificial lighting of 100-150 W/m^2 (ZN-8 lamps). The method of cultivation was hydroponic with feeding by a Knop nutrient solution three times daily. Adjustment of the solution was made every three days for the principal elements of mineral nutrition. The nutrient solution was not changed throughout the test. A keramzit in a fraction of 0.5-1.5 cm was used as a substrate for cultivation of the plants. Certain root crop plants — radishes, carrots, beets and leaf plants — Chinese and white-headed cabbage, were the objects of investigation. We cultivated the plants in companion sowing. The duration of the test was thirty days. Plants with a long vegetational period (carrots, beets, white-headed cabbage) were transplanted once during the test, and plants with a short vegetational period (radishes, Chinese cabbage) were transplanted twice. The amount of yield (Table 1) was determined at the completion of the test. The data obtained were compared with average yields of plants which have been cultivated under greenhouse conditions and under open conditions (Edel'shteyn, 1953; Chesnokov et al., 1960; Moshkov, 1963; Zhurbitskiy, 1963; Bentli, 1965, et al.). The data in the table show that the yield of crops in the test (except for Chinese cabbage) exceeds the level of the average yields of these crops in open and greenhouse soil.

The plants were analyzed for the amount of certain microelements. We used the method of direct spectral analysis in order to analyze the microelements in the moist biomass of the plants.

TABLE 1. AMOUNT OF YIELD OF PLANTS (IN kg/m^2).

Crops and Varieties	Test data	Data from the literature
Nantskaya carrots	19.0	8.0
Bordeaux beets	19.2	7.5
Dunganskaya radishes	19.6	18.0
Chinese cabbage (Khivinskaya reproduction)	16.8	22.5
"Dinosaur" hybrid No. 1X white-headed cabbage	15.6	7.0

The data in Table 2 reveal that there is a marked buildup of ions of heavy /247
metals in the economically useful part and the waste products of plants, while the most intensive accumulation took place in the plantings of Chinese cabbage and white-headed cabbage, while damage to the burner devices was noticed during their mineralization.

The accumulation of the elements under investigation in Chinese and white-headed cabbage exceeded by many times the accumulation in carrots, beets and radishes. The most significant amounts were in the economically nonuseful portion which was mineralized.

TABLE 2. THE AMOUNT OF CERTAIN MICROELEMENTS IN PLANTS. (IN mg PER 100 g OF DRY MATTER).

Crop	Al	B	Fe	Mn	Cu	Zn	Total
Economically useful portion							
Carrots	19,9	6,6	42,7	2,7	0,6	7,4	79,9
Beets	18,4	3,0	17,5	4,5	0,5	—	43,9
Radishes	25,0	3,8	32,8	2,3	0,3	—	64,2
Chinese cabbage	36,3	17,5	40,7	5,0	1,4	—	100,9
White-headed cabbage	129,5	9,7	131,5	3,3	0,8	—	274,8
Economically nonuseful part							
Carrots	8,8	22,7	13,2	9,5	0,8	—	55,0
Beets	24,5	11,2	32,0	13,2	0,4	—	81,3
Radishes	22,9	18,2	24,6	2,6	0,6	—	68,9
Chinese cabbage	157,0	9,7	165,0	8,6	3,8	19,2	363,3
White-headed cabbage	768,0	14,5	750,0	49,5	5,4	25,4	1612,8

Note: Commas indicate decimal points.

The ability of different kinds of plants for selective accumulation of /248
one or another kind of heavy metals (including rare metals) has been known for a long time. V. I. Vernadskiy called them plant-concentrators. It is possible to isolate lithium, zinc, aluminum, and cobalt flora which include plants which are capable of accumulating one or another elements in their tissues.

The entry of elements into plants depends on soil conditions during growing which determine the mineral nutrition of plants. During the cultivation of plants on a porous clay filler (since it is an aluminum-ferrosilicate) ions of Al, Fe and a number of other metals are washed out into solution due to the action of root discharges of the plants. These metal ions may be absorbed and accumulated by the root systems of certain plants. Destruction of the substrates (perlite and vermiculite) due to the action of root emissions was noticed by a number of authors (Yermakov, 1965; Dmitriyeva, Petrov, 1963, et al.). Probably these very processes occur during the use of a porous clay filler, that is a number of heavy metals separate out from the substrate into the nutrient solution due to the action of different products of the metabolism of root systems. The fact that the plants used in companion sowing accumulated microelements in different amounts is proof of the variability of their selective capability in relation to these elements. Plantings of carrots, beets and radishes resisted to a certain extent the buildup of the elements examined because both types of cabbage accumulated them in significant amounts which obviously reflects the specific peculiarities of these plants.

In this matter, we discovered in our tests that plantings of cabbage can accumulate Al, Fe, Mn and Zn in amounts which significantly exceed their accumulation by other cultures (radishes, beets and carrots) due to their biological peculiarities. The accumulation of elements occurs in the entire biomass of plants but is especially concentrated in their root systems. Our examination of data on the total amount of microelements in waste products of a biocomplex shows that root crop plants contain them in an amount from 55 to 81.3 mg for 100 g of dry weight, while cabbage plants contain them in the amount from 363.3 to 1,612.8 mg for 100 g of dry matter. The noticeable buildup of certain microelements (especially of elements with changing valances) is shown by the catalyzing effect during thermal mineralization which can bring about

damage to the oxide film and of course, destruction of the working surfaces of the burners.

In summarizing all of the above, we can establish the fact of a significant buildup of heavy metal in the economically nonuseful part of crops of chinese and white-headed cabbage. Inasmuch as these plants can enter into the human food supply, mineralization of their waste products becomes necessary. This /249 in turn makes necessary a number of additional requirements in the manufacture of burner devices for mineralization of vegetative waste products by the thermal method.

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STUDY OF A METHOD OF CONSERVATION OF URINE RELATIVE TO SPACEFLIGHT CONDITIONS

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ABSTRACT: Seven phenyl-containing substances were studied for purposes of conservation of urine during storage. The most effective seems to be the preparation called "PNF." It easily dissolves in urine and assures its conservation for 100 days under normal conditions of a loss of 0.2 g per 100 ml of urine.

The preparation "PNF," included in the filler of a sanitation device, practically did not change the physical--mechanical properties of the latter but did give the filler an antimicrobial activity.

The conservation of urine in containers of certain types of sanitation /249
devices occupies an important place in a set of measures which are intended to ensure normal conditions for the vital activity of man in the cabin of a space vehicle.

As is known, microorganisms which have entered the urine from the collection systems, from the air and from the membranes of the uro-genital tract, etc quickly multiply during the storage of urine. This can lead to development of toxic products and unpleasant odors in the air of the hermetically-sealed cabin of a space vehicle. Therefore, the urine must preserve relative sterility during prolonged storage.

From sources in the literature it is known that phenyl-containing preparations have a high degree of anitmicrobial activity. Phenyl was authorized as a conservant for medicinal preparations (8 ed. VTUF 2503-58, F 8) by the USSR Pharmacopeia and by its technical properties. Many researchers have made a /250
study of these compounds for disinfection of excreta (Fialkov et al., 1943; Yeveyn, 1893; Okuevskiy, 1932; Bekker, 1948). However, the phenyl-containing preparations have a number of drawbacks — a sharp, irritating odor, high toxicity for warm-blooded animals, and a volatility which makes it difficult to use them under conditions of hermetically-sealed enclosures.

Recently, preparations made from a group of phenyls which have a weak odor and whose staining power has been removed, and whose disinfecting properties exceed those of carbolic acid by 4-5 times and even more (Shakhbazyan, 1961; Bashkov et al., 1957; Vashkov et al., 1960; Sykes, 1958; Erlandson, 1953) have been actively synthesized in our country and have been introduced into the practice of disinfection.

We studied seven phenyl-containing compounds which were prepared at the TsNIDI for the purpose of conservation of urine in the cabin of space vehicles: benzylphenyl, benzylchlorophenyl, phenoltrichloracetate, hexachlorophene, resorcin, ST-2 liquid and PNF. All of these preparations have a weak odor, do not produce precipitates in the urine and do not form additional odors when they enter into chemical reaction with urine components. In addition, they are dechlorizers, do not corrode metals which are often used for sanitation devices and are only slightly toxic to warm-blooded animals (Shakhbazyan, 1961); Mertsalova, 1960; Sykes, 1958; Could et al., 1953; Einola, 1955; Erlandson, 1953).

Freshly eliminated urine, which has been naturally and artificially infected by *Escherichia coli* or by golden staphyllococcus, is poured into sterile flasks in amounts of 100 *ml* and conservants in different amounts are added to it. Then the flasks are placed in storage and from them seedings are periodically made in nutrient media for calculation of the total amount of microorganisms in 1 *ml* of urine. The control samples of the urine (without the addition of preparations) are stored under the same conditions and are subjected to the same tests (Table 1).

It turned out that the preparations, except for ST-2, which were added to the urine in a 1% concentration, prevented its decay for 100 days. The most transparent was the urine into which the preparation of PNF was added as a conservant. It easily dissolved in the urine, had a strong antimicrobial action and delayed multiplication of microorganisms in a 0.05% concentration. The least effective were the phenyltrichloracetate and the ST-2 liquid.

TABLE 1. CONSERVATIVE EFFECT OF PHENYL-CONTAINING PREPARATIONS ON URINE DURING ITS PROLONGED STORAGE. /251

Preparations	Prep. in urine, %	Results of bacteriological study (exposure time in days)						
		1	5	10	15	30	60	100
Preparation of PNF	0,02	+	+	++	+	+	+	+
	0,05	—	—	—	—	—	—	—
	0,1	—	—	—	—	—	—	—
	1	—	—	—	—	—	—	—
Benzylphenyl	0,1	+	+	+	+	+	+	+
	0,2	—	—	—	—	—	—	—
	0,5	—	—	—	—	—	—	—
	1	—	—	—	—	—	—	—
Benzylchloro-phenyl	0,1	+	+	+	+	+	+	+
	0,2	—	—	—	—	—	—	—
	0,5	—	—	—	—	—	—	—
	1	—	—	—	—	—	—	—
Hexachlorophene	0,1	+	+	+	+	+	+	+
	0,3	+	+	+	+	+	+	+
	0,4	+	+	+	+	+	+	+
	0,8	+	+	+	+	+	+	+
	0,9	+	+	—	+	+	—	+
	1	—	—	—	—	—	—	—
Phenyltrichlor-acetate	0,2	+	+	+	+	+	+	+
	0,5	—	—	—	—	—	—	—
	1	—	—	—	—	—	—	—
Resorcin	1	—	—	—	—	—	—	—
Preparation ST-2	0,5	+	+	+	+	+	+	+
	1	+	+	—	—	—	+	+
	5,0	—	—	—	—	—	—	—

Note: commas indicate decimal points.

In working with urine which has been artificially infected by strains of *Escherichia coli* and golden staphyllococcus which are resistant to phenyl (1,000 microbial bodies in 1 ml), the smallest dose of PNF which ensures preservation of urine for 100 days is 0.2 g for 100 ml of urine.

The benzylchlorophenyl, benzylphenyl and resorcin were also effective but /251
they had a slight odor which was maintained during prolonged storage of urine
with the substances. Therefore, use of the latter in spaceflight conditions
may be admitted only after careful study of the gas composition of the odorific /252
substances and their subsequent sorption.

At the present time, fillers which have antimicrobial activity that our data indicate is insufficient for conservation of urine during its storage are used in certain designs of sanitation devices intended for space vehicles. Regarding this matter, we studied the possibility of using the conservant PNF for these purposes by administering it into the composition of the filler of a sanitation device of a space vehicle. It gave stable antimicrobial properties to the latter and suppressed multiplication of microorganisms in the urine for a long time (more than 30 days).

Study of the physical-mechanical properties of the filler showed that during its treatment with the indicated conservant it had a somewhat smaller actual moisture content (by 0.9%), however, the amount of this deviation is so small that in practice its properties did not change (Table 2).

TABLE 2. MOISTURE OF SAMPLES OF A FILLER WHICH HAS BEEN TREATED AND REPROCESSED BY THE PNF CONSERVANT.

Filler	Weight before drying, g (M ± m)	Weight after drying, g (M ± m)	Moisture, %
Without treatment	2.002 ± 0.00002	1.9112 ± 0.0008	4.6
With treatment	2.0015 ± 0.01	1.9310 ± 0.006	3.7

The absorptive capacity of the samples of the urine fillers, which have been treated with PNF, also decrease insignificantly in comparison with the starting value of this index (by 0.8-0.6%) (Table 3).

TABLE 3. ABSORPTIVE CAPACITY OF SAMPLES OF FILLERS WHICH HAVE BEEN TREATED WITH THE PNF CONSERVANT OR NOT.

Type of filler	Treatment	Absorptive capacity, g (M ± m)
No. 1	Without treatment	19.2 ± 0.42
	Treated	18.4 ± 0.35
No. 2	Without treatment	10.1 ± 0.08
	Treated	9.1 ± 0.11

We did not notice an essential difference in the indices of the capillary rise of fluie in samples of fillers which had been treated and not treated with this antiseptic (Table 4).

TABLE 4. CAPILLARY RISE OF FLUID IN SAMPLES OF FILLERS WHICH HAVE BEEN TREATED WITH THE PNF CONSERVANT AND THOSE WHICH HAVE NOT.

Filler	Rise time, seconds			
	5	15	30	60
	Height, mm			
Without treatment	22.4 ± 0.5	36.1 ± 1.0	50.2 ± 1.0	68.5 ± 1.6
With treatment	22.3 ± 0.5	41.2 ± 1.4	55.7 ± 2.2	70.1 ± 1.3

Continuation of Table 4

Filler	Rise time, seconds			
	120	180	240	300
	Height, mm			
Without treatment	88.3 ± 1.7	90.2 ± 1.1	95.1 ± 1.7	97.2 ± 1.0
With treatment	82.3 ± 1.0	89.7 ± 1.5	93.5 ± 1.4	95.2 ± 1.0

Our studies lead us to the conclusion that the PNF conservant is compatible with substances which absorb the urine, which are used in designs of certain kinds of sedimentation devices of space vehicles, and it can be recommended for further tests in hermetically-sealed chamber experiments. /253

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THE FOOD COMPONENT, BASED ON STORES OF DEHYDRATED PRODUCTS, IN LIFE-SUPPORT SYSTEMS FOR CREWS OF SPACE VEHICLES DURING PROLONGED FLIGHTS

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ABSTRACT: Tests were conducted on the state of health, the metabolic processes and the immunoreactivity of the organisms of six test subjects who were kept on a ration of dehydrated food products for 120 days.

The data obtained in the experiment for the level of elimination of a number of substances from the organisms and for the balance of certain elements may be used in calculating the food component, which is based on stores of dehydrated products, in prolonged flight with daily energy consumptions of about 3,000 kcal on the part of the cosmonauts. Adaptation of the organism to such a diet occurs in the first two months.

The use of stores of dehydrated products is a realistic way of solving the /254
problem of nutrition in spaceflights. This means would also be the only one possible as long as the weight of any other system for producing food exceeds the weight of the reserves for the duration of a flight.

In order to determine the possibility of using dehydrated products for nutrition, we studied the effect of diets which included them on the exchange processes of an organism. Foreign researchers also have conducted such studies (Gerathewohl, 1963; Welch et al., 1961; Speckmann et al., 1965; Heidelbaugh et al., 1966; Smith et al., 1966), as well as Soviet scientists (Karandayeva, 1965; Ivanov et al., 1964; Bychkov et al., 1966). As a result of these studies lasting from 17 to 56 days, we determined that significant deviations in metabolism in the state of the health of the test subjects were not present.

The present studies on six volunteer test subjects were undertaken in order to determine the possibility of a more prolonged nutrition based on a diet of dehydrated food products, in order to study the effect of this diet on metabolism and reactivity of the human organism, and also in order to obtain

starting data which are necessary for calculation of the food component, which is based on stores in life-support systems for crews of space vehicles during prolonged flight.

In studies conducted from December 1966 through May 1967 the test subjects were fed a diet composed of dehydrated products. The dehydrated products were rehydrated before preparation of the food. The background nutrition diet (in May and December) was similar to the test ration in chemical composition and selection of products.

The test subjects (men) underwent critical examination in November 1966 and were admitted to participation in the experiments. Brief characteristics of the test subjects are given in Table 1.

TABLE 1. CHARACTERISTICS OF THE TEST SUBJECTS.

Test subject	Age	Height, cm	Weight, kg	Ideal wt. (Lester, 1949)
V-v	23	179	79	72.3
D-k	31	172	87	69.6
K-n	31	166	73	64.1
M-v	28	179	91	74.6
P-v	31	170	76	67.7
S-v	28	172	68	68.0

The test subjects carried out their official duties and maintained a normal cycle of work and rest. During the last week of each month they stayed in the laboratory for a full 24-hour period under constant observation by /255 physicians. The metabolism of the test subjects was studied on these days.

Each ration was composed of a three-day menu with four meals a day. The food was prepared by an expert cook.

The amount of nutritive substances and the caloric content of the food ration which was composed of natural and dehydrated products are given in Table 2.

TABLE 2. COMPOSITION AND CALORIC VALUE OF THE FOOD RATION. (ACCORDING TO ANALYTICAL DATA).

Days of menu	Overall weight, g	Water, g	Proteins, g	Fat, g	Carbo-hydrates, g	Caloric value, kcal
			Natural products			
1st	2,016	1,347	125	93	435	3,160
2nd	1,930	1,240	137	99	430	3,250
3rd	1,940	1,280	127	100	407	3,131
Mean daily ration	1,960	1,289	130	97	424	3,180
			Dehydrated products			
1st	714	56	124	90	424	3,085
2nd	717	47	134	98	425	3,200
3rd	660	44	126	101	384	3,032
Mean daily ration	697	52	128	96	411	3,105

From the table it is evident that the weight of the mean daily food ration composed of dehydrated products was ~700 g. The ratio of proteins, fats and carbohydrates in the ration was 1:0.75:3.2, that is, it was close to the accepted level for persons who are not engaged in physical work (Molchanova, 1951, 1953; Pokrovskiy, 1966).

Proteins of animal origin were 64% of the proteins in the ratio. Vegetable /256 fats were 40% of the fats in the ration, that is the amount of animal proteins and vegetable fats was optimum.

The rations also contained a sufficient amount of irreplaceable amino acids (Table 3).

The amount of vitamins used in the daily rations (based on calculated data) somewhat exceeded the accepted norms for these vitamins (Table 4).

The amount of basic mineral elements in the average daily food rations is given in Table 5.

TABLE 3. AMOUNT OF IRREPLACEABLE AMINO ACIDS IN THE DAILY FOOD RATIONS (CALCULATED DATA).

Amino acids	Amount, g				
	1st day	2nd day	3rd day	Mean daily amount	Mean daily requirement (Pokrovskiy, 1964)
Threonine	4,6	5,0	4,8	4,8	2—3
Isoleucine	5,7	6,2	5,9	5,9	3—4
Leucine	9,6	10,3	10,1	10,0	4—6
Lysine	7,1	8,9	7,5	7,8	3—5
Methionine	2,4	2,4	2,3	2,4	2—4
Phenylalanine	5,3	5,6	5,7	5,5	2—4
Tryptophan	1,4	2,9	2,2	2,2	1,0
Valine	6,5	6,2	6,0	6,2	4,0

Note: Commas indicate decimal points.

TABLE 4. AMOUNT OF VITAMINS IN THE DAILY FOOD RATION (CALCULATED DATA).

Vitamins	Amount, mg				
	1st day	2nd day	3rd day	Mean daily amount	Mean daily requirement, (Pokrovskiy, 1964)
Vitamin A	0,690	0,638	0,632	0,653	1,5—2,5
Carotene	13,815	9,435	13,955	12,4	
Vitamin B_1	3,815	4,761	3,371	4,0	1,5—2,0
Vitamin B_2	4,117	4,422	3,832	4,1	2,0—2,5
Vitamin PP	41,649	43,826	40,006	42,0	15—25
Vitamin C	207,47	145,30	218,25	190,0	70—100

Note: Commas indicate decimal points.

TABLE 5. AMOUNT OF MINERAL ELEMENTS (IN g) IN THE AVERAGE DAILY FOOD RATION (ANALYTICAL DATA).

Element	Ration			
	Background	Test	Regenerative period	Norm (Pokrovskiy, 1964)
Potassium	3,83	3,13	4,39	2,5—5,0
Calcium	1,02	0,82	1,03	0,8—1,0
Magnesium	0,54	0,53	0,63	0,3—0,5
Sodium	6,38	7,08	6,72	4,0—6,0
Chlorine	9,89	10,92	13,08	5,0—7,0
Phosphorus	2,12	2,03	2,22	1,0—1,5

Note: Commas indicate decimal points.

Evaluation of the food ration was made on the basis of a study of the following indices: /257

--Chemical composition of the ration (amount of nutrient substances, mineral elements, water);

--organoleptic properties of the products;

--monthly clinical examination of the test subjects (functional condition of the stomach, liver, clinical analyses of the blood and urine, adaptation of the eyes to darkness);

--daily medical observation of the test subjects (weighing, temperature reading, measurement of blood pressure, physical examination);

--study of the level of protein metabolism (in blood serum — total protein, protein fractions, free amino acids, residual nitrogen, urea; in the urine — total amine nitrogen, amino acids, urea, ammonia, uric acid (creatine, creatinine,; in perspiration and in the ration — total nitrogen; nitrogen balance; assimilation of protein);

--study of the level of lipid metabolism (in blood serum — total lipids, lipoproteids, phosphorus of phospholipids, total and esterified cholesterin; amount of fats in the ration and in the perspiration; assimilation of fat);

--study of the level of carbohydrate metabolism (tolerance of an organism for glucose, amylase in the blood serum; diastasis — in the urine; carbohydrates — in the ration and in perspiration; assimilation of carbohydrates);

--metabolism of vitamins (in blood serum — vitamins A, E, carotene; in the urine — vitamins B_1, B_2, C, 4-pyridoxinic acid);

--mineral metabolism (in blood serum — amount of sodium, potassium, magnesium, calcium, chlorine, phosphorus, sulfur, — once a month; the same elements in the urine — daily — the same elements in perspiration and in food rations; balance and assimilation of these elements);

--water metabolism (quantity of liquid entering the organism in the form of drinks of water included in the ration; calculation of the amount of metabolic water; daily calculation of the volume of urine; study of the moisture /258

in perspiration on the exchange days; determination of the volume of intracellular fluid according to the concentration of glucose in blood serum);

--basic metabolism (by a method of the gas exchange study on the "Spirolit" apparatus);

--study of the immune reactivity of an organism (phagocytic activity of leucocytes in the peripheral blood; concentration of salivary acids in blood serum, lysozyme activity of saliva and blood serum; microflora of the oral cavity and throat; bacterial flora of the intestines);

--determination of gas-like micromixtures in expired air — monthly (ammonia and amines, aldehydes, alcohols, dimethylamine, carbon monoxide).

The data obtained were subjected to statistical analysis according to the St'yudent-Fisher method (Kaminskiy, 1959).

The last six days at the end of each month were exchange days as we have indicated above. On these days the test subjects were kept in the laboratory around the clock. During these six days we collected their perspiration, and isolated portions from the first and last day by "samples" of carmine. The perspiration of each test subject was dried by the lyophilic method, crushed and analyzed for the amount of moisture, nitrogen, fat, ash, and mineral elements. The food ration for three days was dried, crushed, and analyzed in the same way as was the perspiration.

The test subjects' urine was collected and analyzed daily on the same days. At the end of this period 70 mℓ of blood were taken from the veins of each test subject in order to determine the indices mentioned above.

As a result of the studies we determined that the food ration composed of dehydrated products was evaluated positively in its organoleptic properties by the test subjects. The test subjects ate all of the test rations. Only a few dishes (vegetable stew, et al.) were given low marks. In any case, the food ration of natural products (background) received much higher praise than the test ration from all of the test subjects.

The dynamics of body weight of the test subjects throughout the experiment (by month) is given in Table 6.

TABLE 6. DYNAMICS OF BODY WEIGHT OF THE TEST SUBJECTS (IN kg).

Test subject	Starting level	January	February	March	April	May	Change in wt.		Ideal weight
							kg	%	
V-v	78,4	77,5	76,2	75,9	76,0	75,9	—2,5	—3,2	72
D-k	86,2	86,4	86,6	86,9	86,0	86,2	0	0	69
K-n	72,1	72,4	72,5	72,5	72,7	72,4	+0,3	+0,4	64
M-v	87,5	86,5	86,9	86,5	84,3	80,0	—7,5	—8,6	74
P-v	74,6	74,2	73,7	73,7	74,4	73,5	—2,5	—3,3	67
S-v	69,9	69,3	69,8	69,8	70,5	70,0	+0,1	+0,1	68

Note: Commas indicate decimal points.

In the table it can be seen that the weight for the three test subjects in comparison with the background data did not change, but in three others it decreased, especially pronounced in the test subject M-v during April and May. This circumstance is related primarily to the fact that this test subject had a significant amount of excess weight, and secondly, during these months he was taking state examinations and was finishing his degree work.

The test subjects who had a significant amount of excess weight and a /259
starting weight of more than 75 kg also lost weight during the experiment. The calorie value of the tested food ration was insufficient for them.

Certain functional changes in the activity of the gastro-intestinal tract (heartburn after eating, dryness in the mouth) were noticed in test subjects during feeding of the ration of dehydrated food products. The secretive function of the stomach was studied by means of fractional probing by a thin probe; we used a cabbage broth as a stimulator of the gastric glands. We studied the amount of gastric juice obtained during the hour following injection of cabbage broth, that is, "hourly stress;" we determined fractionally the acidity and the amount of free hydrochloric acid in titration units.

We noticed changes throughout the experiment in the secretion of gastric juice, both increases and decreases. We noticed a tendency in all the test subjects to an increase in total acidity and free hydrochloric acid in the gastric juice (Tables 7 and 8), especially during the second month by feeding with a test ration which confirms the excitation of the acid-forming function of the stomach in this period. At the end of the third month the acidity of the gastric juice began to decrease, but by the end of the fourth month it almost returned to the starting value level.

These changes can be explained by the process of adaptation of the digestive glands to the ration of hydrated food products. The duration of the adaptation period is evidently related to the adjustment of the enzyme systems which participate in digestion.

TABLE 7. TOTAL ACIDITY OF GASTRIC JUICE IN TEST SUBJECTS.

Test subject	Period					
	Starting	Test				Regenerative
	December	January	February	March	April	May*
V-v	112	120	118	138	106	116
D-k	80	106	116	130	116	106
K-n	92	86	114	110	80	--
M-v	130	116	170	152	114	110
P-v	86	88	130	90	96	112
S-v	54	88	118	44	78	90
M	92	100	128	111	98	107
t	--	0.7	2.7	1.0	0.5	1.1

*Data for May were compared with April.
Note: Commas indicate decimal points.

During the first month of the test period we noticed an increase in the amount of total and direct bilirubin and a reduction in the amount of cholinesterase in the blood serum. Although the fluctuations of these indices

did not go beyond the limits of the norm, they evidently are related to changes in the functional state of the liver. Toward the end of the second month of the test period these indices were almost indistinguishable from the background indices. /260

TABLE 8. AMOUNT OF FREE HYDROCHLORIC ACID IN GASTRIC JUICE OF THE TEST SUBJECTS.

Test subject	Period					
	Starting	Test				Regenerative
	December	January	February	March	April	May
V-v	88	100	98	120	90	92
D-k	60	88	94	114	104	84
K-n	66	66	98	94	60	--
M-v	116	100	156	140	100	90
P-v	70	72	114	76	84	94
S-v	38	66	100	28	62	72
M	73	82	110	95	83	86
t	--	0.7	2.6	1.1	0.7	0.3

Note: Commas indicate decimal points.

Clinical analyses of the urine and blood throughout the entire experiment showed no deviations from the normal. We noticed no statistically verifiable differences in the amount of total protein in blood serum throughout the entire experiment in comparison to the background data. We noticed only a pronounced tendency towards reduction of this index in February, April and May (Table 9).

The average amount of albumin fraction in blood serum of the test subjects increased from December (background) through March (third month of the test) from 5.30 g% in December to 5.90 g% in March. In April-May the amount of albumins returned to the starting value. The globulin fractions of blood serum (α_1, α_2, β, γ) in all the test subjects decreased sharply in February (second month of the test) and this reduction was statistically reliable for all of the fractions (except γ). In March (third month of the test) we noticed a clear tendency towards normalization of these indices. The amount of the globulin fractions in the blood serum in February (second month of the test) was below normal. The greatest increase of A/G coefficient in February is /262

explained by the increase of albumin by this time and the reduction of the globulin fraction of the blood serum proteins.

Changes of the protein fractions of the blood serum evidently indicate that the changes in the functional state of the liver occur during feeding with the test ration, as these changes bring about the synthesis of proteins of the blood serum. The processes of readjustment of the liver function to the new conditions were most pronounced by the end of the second month of the test period. A clear tendency towards normalization of the change indices of metabolism showed up by the end of the third month.

Concentration of the majority of the tested free amino acids in the blood serum of the test subjects during the test period was lower in comparison to the background and regenerative periods. A decrease in concentration of amino acids in the blood serum of the test subjects during the test period evidently occurred due to the more difficult digestibility of proteins of dehydrated products by proteolytic enzymes of the gastro-intestinal tract. During dehydration of the products the structure of the protein is changed and protein and protein-carbohydrate complexes are formed which are subjected to the action of enzymes in the gastro-intestinal tract with dissimilar rates. This can lead to delay in liberation and in absorption of certain amino acids. As a result of this the amino acid entered the liver and other organs in which synthesis of protein takes place in an insufficiently balanced ratio and therefore they were eliminated with the urine in a greater amount than in normal nutrition. This is shown by the increased elimination of amine nitrogen along with the urine at this time. The level of the majority of amino acids in the blood serum began to increase by the end of the test period, which is confirmation of the increasing adaptation of the organisms to the ration of dehydrated food products at this time.

Studies of the attacking of proteins of dehydrated food products by proteolytic enzymes confirms the theory that we have mentioned.

TABLE 9. AMOUNT OF PROTEIN AND AMINO ACIDS IN BLOOD SERUM OF TEST SUBJECTS AT DIFFERENT PERIODS OF THE EXPERIMENT.

Indices	M	t	M	t	M	t	M	t	M	t
	XII–I	XII–I	II	XII–II	III	XII–III	IV	XII–IV	V	XII–V
Total protein and protein fractions of blood serum, g%										
Total protein	8,12	8,11	0,03	7,82	1,07	8,17	0,19	7,50	2,30	7,45	0,29
Albumins	5,30	5,51	1,5	5,87	3,8	5,90	4,3	5,31	0,03	5,46	0,7
Globulins:											
α_1	0,32	0,32	0	0,17	8,6	0,22	4,5	0,19	5,9	0,15	1,8
α_2	0,56	0,48	2,0	0,36	6,6	0,42	3,5	0,43	2,8	0,45	0,4
β	0,80	0,73	2,3	0,56	6,6	0,65	2,0	0,66	2,6	0,59	1,4
γ	1,04	1,07	0,27	0,86	1,6	0,98	0,46	0,91	1,1	0,80	1,1
A/G	2,0	2,14	1,0	3,05	5,8	2,66	3,3	2,52	1,6	2,75	0,75
Free amino acids in blood serum, mg/100 m										
Cystine	1,43	1,00	1,34	1,08	1,30	1,14	0,90	0,97	1,84	2,00	2,64
Lysine + histidine	2,32	1,75	3,56	2,39	0,20	2,26	0,31	2,47	0,53	3,12	1,54
Arginine	0,98	0,57	3,72	0,45	3,78	0,59	2,16	0,49	5,76	0,85	4,00
Asparaginic acid + serin + + glycine	4,60	3,66	2,04	3,40	3,00	3,21	3,97	3,70	2,25	4,16	1,58
Glutamic acid + threonine	2,31	1,65	5,07	1,70	5,54	1,61	5,00	1,96	1,96	2,15	1,12
Alanine	1,77	1,61	0,80	1,79	0,12	1,56	1,17	1,96	0,70	2,20	0,92
Tyrosine											
Methionine + valine	0,68	1,46	4,33	0,76	0,44	0,77	0,47	1,20	1,89	1,15	0,06
Phenylalanine + leucine + + isoleucine	3,35	1,89	9,13	2,38	8,08	1,87	10,57	3,30	0,09	3,00	0,50
	2,49	2,70	0,70	2,16	1,14	1,29	4,00	1,60	1,89	2,27	1,63

Note: Commas indicate decimal points.

The elimination of amine nitrogen in the urine (Table 10) during the test and regenerative periods of the investigation was higher in comparison with the background. These differences were statistically reliable in March and April. The remaining end products of protein metabolism did not undergo any essential changes. /263

TABLE 10. AMOUNT OF AMINE NITROGEN IN THE URINE OF TEST SUBJECTS AT DIFFERENT PERIODS OF THE EXPERIMENT.

Test subject	Background	Test				Regenerative
	December	January	February	March	April	May
V-v	0,44	0,47	0,50	0,54	0,54	0,57
D-k	0,34	0,46	0,43	0,54	0,47	0,52
K-n	0,25	0,34	0,41	0,48	0,48	0,53
M-v	0,39	0,43	0,45	0,50	0,51	0,46
P-v	0,40	0,54	0,53	0,62	0,57	0,70
S-v	0,36	0,36	0,39	0,60	0,36	0,39
M	0,36	0,43	0,45	0,55	0,49	0,53
t at P = 95%	—	1,7	2,2	4,7	3,2	0,8

Note: Commas indicate decimal points.

Assimilation of protein from the background and test food rations fluctuated on the average from 86 to 89%.

Regular changes of the indices of lipid metabolism (common lipid; common, esterified, and free cholesterin; phosphorus of phospholipids; α- and β-lipoproteids in the blood serum) did not occur throughout the entire experiment. Their amounts were found to be at the normal level (Table 11).

Assimilation of fat of the background and test food rations fluctuated throughout the experiment from 93.3 (in the background) to 96.4% (in the third month of the test) and was close to the average assimilation of fat during feeding on a mixed ration of natural food products. There was some tendency toward increasing of assimilation of fat during the test and regenerative periods in comparison with the background period which might be explained by the adaptation of the digestive glands to the ration of the given chemical composition.

TABLE 11. CERTAIN INDICES OF THE LIPID, CARBOHYDRATE AND VITAMIN METABOLISMS ACCORDING TO MONTHS.

Indices	December	January	February	March	April	May
Lipid metabolism (in blood serum)						
Common lipids, mg %	557±67*	574±77	577±80	545±68	613±77	530±82
Common cholesterin, mg %	220±31	232±25	205±24	178±35	186±33	179±28
Esterified cholesterin, mg %	179±58**	167±24	152±24	127±22	140±31	132±18
Free cholesterin, mg %	54±16	65±17	57±22	52±20	46±18	47±17
Esterification, %	78±10	71±7	73±10	70±4	76±8	74±6
Phosphorus of phosphatides, mg %	5,6±0,4	5,6±0,7	5,4±0,4	6,7±0,9	6,6±0,2	6,0±0,6
-lipoproteids, %	21,6±3,0	23,8±5,7	22,1±4,5	20,7±4,2	22,5±2,7	23,3±2,2
-lipoproteids, %	78,4±3,0	76,2±5,7	77,9±4,4	79,3±4,2	77,5±2,7	76,7±2,2
Carbohydrate metabolism						
Blood sugar, mg %	91±13	—	—	89±18	104±15	—
Coefficient of assimilation of glucose by tissues	$2,5 \cdot 10^{-2} \pm 0,8 \cdot 10^{-2}$	—	—	$2,7 \cdot 10^{-2} \pm 0,8 \cdot 10^{-2}$	$2,3 \cdot 10^{-2} \pm 0,8 \cdot 10^{-2}$	—
Blood amylase, %	16±5	—	18±1	17±2,6	16±2,6	14,5±2,5
Vitamin metabolism (in blood serum)						
Vitamin A, ME in 100 m	148±31	210±19	164±20	201±43	201±43	175±17
Carotene,	43±9	58±16	38±12	50±19	79±20	88±25
Vitamin metabolism (in urine)						
Vitamin B_1, µg/hr	18±3	23±7	28±8	29±7	18±6	23±4
Vitamin B_2, µg/hr	79±25	80±19	84±22	101±27	104±47	92±33
Vitamin C, µg/hr	1,3±0,35	0,9±0,2	1,5±0,5	1,3±0,3	1,1±0,1	1,5±0,3

* M + mtp 95.

** Average indices of cholesterin, esterified in December, were computed for the data of four test subjects, and for five test subjects in the month of February.

Note: Commas indicate decimal points.

The indices of the state of the carbohydrate metabolism (tolerance of the organism to glucose, coefficient of assimilation of glucose by tissues, blood amylase) stayed within normal limits throughout the entire experiment (Table 11). Assimilation of carbohydrates was practically the same and fluctuated within limits from 96 to 97%.

We determined the provision of the organism of the test subjects with vitamins according to their elimination in the urine and according to the level /264
of their amount in the blood (Table 11). The amount of vitamin A and carotene in the blood serum of the test subjects was normal throughout the entire test. During the test period the concentration of these vitamins and vitamin E was higher than in the background period. The excretion of vitamins B_1, B_2 and 4-pyridoxinic acid stayed at normal levels, but the excretion of vitamin C, which began in February, was somewhat higher than the normal. This can be explained by the increased amount of this vitamin in the food rations.

In spite of the identical entrance of fat soluble vitamins into the organism of test subjects along with the background and test food rations, their amount in the blood serum during the test period was significantly higher. In order to explain the causes of this phenomenon, special investigations must be conducted.

The amount of mineral elements studied by us (calcium, potassium, magnesium, phosphorus, sodium and chlorine) in the blood serum of the test subjects remained at a normal level throughout the entire test (according to data of A. P. Tereshchenko, Ye. I. Pokrovskaya et al.). Assimilation of these elements (except for calcium) in the background and in the test differed only slightly from one another. The assimilation of calcium in the test period decreased, especially noticeably during the last two months (March and April). Additional investigations must be conducted in order to provide a final explanation of the peculiarities of assimilation of calcium during feeding with a ration of dehydrated products.

The water balance of the test subjects at various periods of the investigation was different.

Indices of the volume of extracellular fluid (average for the whole group of test subjects), expressed both in liters and in percentages of body weight, did not change in comparison with the starting data.

Thus, the amount of extracellular liquid in the organism of the test subjects did not undergo substantial changes in spite of the changes in the level of the water balance at different periods of the investigation. These data confirm the absence of disturbances of water metabolism in the test subjects during prolonged feeding with a ration of dehydrated products.

The level of basic metabolism in the first two months of the test period (January, February) did not differ from the background. In the last two months of the test period (March, April) the basic metabolism in all of the test subjects decreased; this is evidently explained by seasonal fluctuations in the level of metabolic processes in organisms.

The lysozyme activity of the saliva underwent the following changes in the course of the experiment: in the background — normal, in the first two months of the test period there was a statistically verifiable reduction in activity, /266 in March (third month) — a rise to the level of the starting values (at the end of April (fourth month) — a reduction even in this level before the end of the experiment. A certain reduction in the lysozyme (bacteriolytic) activity of the blood serum was determined only at the end of the first month of feeding on a ration of dehydrated products.

During nutrition on a diet of dehydrated food products over a four-month period we found that there was a pronounced increase of microbes in the oral cavity in the month of January (first month of the test) and especially in February (second month of the test). *Escherichia coli* was seeded in February in all of the test subjects. This quantity decreased in March (third month of the test). In May (regenerative period) the quantity of *Escherichia coli* in the oral cavity increased again in all of the test subjects, except for K-n. The amount of staphylococci was increased both in February and in March (second and third months of the test).

The bacterial composition of the perspiration did not undergo substantial changes during the experiment. We detected no seasonal differences in the quantitative ratio of the basic groups of bacteria in the perspiration.

The phagocytic activity of the leucocytes in the background and in the first month of the test period did not undergo substantial changes. We detected a verifiable increase of this index only during the last month of the test period (in April) and during the regenerative period (May). This increase can be explained by seasonal fluctuations of this index.

We noticed no verifiable changes in the level of the salivary acids in the blood serum during the entire experiment.

Nutrition of a period of four months on a diet of dehydrated products did not lead to quantitative or qualitative changes in the composition of the expired air in comparison with these indices as obtained during nutrition on a diet of natural food products. This can be explained by the similar chemical composition of the background and test food rations.

Balance and Level of Elimination of Certain Substances and Elements in the Test Subjects

The test subjects eliminated the following amount of perspiration during different periods of the experiments: in the background — 183.6, 183.4 in the test period, and 163.1 g in the period of regeneration (average daily data for the six test subjects for each of the mentioned periods are given). Dry substances in the presented amounts of perspiration comprised — 43.2 g in the background, 44.8 g in the test period, and 40.1 g in the regenerative period. Along with the perspiration of the test subjects the following respective amounts were eliminated in the background, test and regenerative periods: 2.34, 2.37 and 2.73 g of nitrogen; 6.38, 4.55, and 4.78 g of fat; 16.75, 20.90 and 13.86 g of carbohydrates; 5.41, 4.52 and 4.32 g of ash; 188.0, 188.5 and 171.0 large calories.

The water balance of the test subjects was ~3,024 m in the periods between the metabolic days on the average and ~2,987 m on the metabolic days. This is probably explained by the somewhat greater activity of the test subjects during the periods between the metabolic days. As a result of the latter, in

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the periods between the metabolic days there was a redistribution of elimination paths for fluid from the organism in addition to a certain increase in the level of the overall water balance (by 37 ml), namely: on the metabolic days 75 ml more of water (1,184 and 1,259 ml) was eliminated along with the urine, and 110 ml less (1,698 and 1,588 ml) was eliminated by the lungs and skin. Approximately 1 ml of liquid was eliminated for each assimilated large calorie of food ration.

The average daily elimination of certain elements in the urine and perspiration of the test subjects during the test period (in g/days) and the limits of their fluctuations are given in Table 12 (data obtained jointly with A. P. Tereshchenko, Ye. I. Pokrovskaya et al.).

TABLE 12. AVERAGE DAILY ELIMINATION (IN g/DAYS) OF CERTAIN ELEMENTS IN URINE AND PERSPIRATION.

Elements	Avg. amt.	Limits of fluctuations	Elements	Avg. amt.	Limits of fluctuations
	Urine			Perspiration	
Sulphur	0.91	0.5 — 1.2	Sulphur	0.50	0.40 — 0.60
Phosphorus	0.97	0.7 — 1.2	Phosphorus	0.86	0.73 — 0.98
Potassium	2.48	1.0 — 2.5	Potassium	0.65	0.55 — 0.71
Calcium	0.15	0.1 — 0.2	Calcium	0.68	0.58 — 0.81
Magnesium	0.12	0.05 — 0.15	Magnesium	0.35	0.30 — 0.40
Sodium	5.10	4.0 — 7.0			
Chlorine	7.19	5.0 — 9.0			

Note: Commas indicate decimal points.

The nitrogen balance (in g/days) is given in Table 13 with calculation of the Grindli correction (Petrun'kina, 1961).

The data in Table 13 shows that the amount of protein contained in the background and test food rations (130 and 128 g, respectively) chiefly insured maintenance of the nitrogen balance in the test subjects during the entire experiment.

The balance of magnesium, phosphorus, sodium and chlorine remained positive throughout the entire experiment. The potassium balance remained positive

for the first, second and fourth months of the test period. In the third month of this period there was a sharp negative potassium balance with losses of up to 1-2 g a day. The latter was caused by the elimination of potassium in the urine, which was observed only during the period when metabolic investigations were being conducted (23-30.03 66 g) and were not observed on the /268
remaining days of this month. The calcium balance in the second half of the test period (third and fourth months) was, as a rule, negative.

TABLE 13. NITROGEN BALANCE AT DIFFERENT PERIODS OF THE INVESTIGATION (IN g/DAYS).

Test subject	Background	Test				Regenerative
	December	January	February	March	April	May
V-v	-2.44	+0.94	+1.74	+0.11	-1.27	+3.47
D-k	-0.32	+1.54	+3.15	+1.54	+3.01	+2.34
K-n	+3.86	+4.55	+5.05	+3.13	+2.84	+4.52
M-v	+0.24	+1.54	+2.07	+0.84	-0.06	+3.22
P-v	+0.64	-0.62	+1.46	-0.26	-0.27	-0.69
S-v	+1.59	+2.75	+2.53	+3.74	+1.72	+4.18
M	+0.58	+1.82	+2.52	+2.28	+0.97	+2.99
t at P = 95%	--	1.50	2.40	1.90	0.44	3.01

Using the above method the data obtained in the experiment for the level of elimination of a number of substances from the organism and for the balance of certain elements can be used in calculating the food component which is based on the stores of dehydrated products in prolonged flights with daily energy consumptions on the part of astronauts of about 3,000 large calories.

Results of an investigation of the state of health, of the metabolic processes and of the immunoreactivity of the organism in test subjects who were fed a ration of dehydrated food products for a period of 120 days showed that adaptation of an organism to such a ration occurs basically during the first two months.

During the 120-day nutrition on a diet of dehydrated food products having a calorie value of about 3,000 large calories, test subjects who had an initial weight of more than 75 kg lost weight during the experiment.

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VI. EXOBIOLOGY

EVOLUTION OF THE PROBLEM OF DETECTING ORGANIC MATTER IN THE SOLAR SYSTEM

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ABSTRACT: This chapter examines the origin of organic compounds which are basic for the primary forms of life. The problem of detection and identification of organic matter in the Solar System is directly connected with the problem of evolution of cosmic organic matter. A single plan is proposed for the evolution of organic matter which includes its biogenic and abiogenic forms. A comparison of the elemental and isotopic composition and the composition of pigments, optical activity, polymerization and cross-linking in the principal strains of evolution of organic matter in the solar system is included.

The problem of detecting extraterrestrial life presents biologists with /270
demands which principally differ from those with which it had to deal during the classical period of its development. It can be said that biology is an authentically theoretical science only within the bounds of this problem.

In contrast, for example, to physics or mathematics, biology has always studied the particularly individual phenomena limited by narrow (on a cosmic scale) bounds. The impossibility of validating how life could develop in conditions different from those of Earth goes beyond the subject of biology — the biosphere in its totality as something unique and inimitable — and brings biology closer to humanitarian sciences than to physical and mathematical natural science.

Discovery of extraterrestrial life must give experimental indications that living organisms are a result of their random habitation under given cosmo--ecological conditions, and that they are related to general principles inherent in any living matter no matter where it originated. However, in order to correctly interpret these indications we must examine evolution of the biosphere as one of the stages of evolution of organic matter of the universe. Therefore

the problem of discovery of extraterrestrial life is closely related to the problems of the origin and development of organic compounds in cosmic space at different stages of the formation of interstellar matter and proto-planetary concentrations under specific conditions of the development of planets.

The introduction of life on Earth should be viewed in relation to the /271
total evolution of the planet, which has given birth to two forms of existence of organic matter, living and nonliving (Bernal, 1966; Oparin, 1966).

To the first group belong individual living organisms, which are more or less clearly delineated from one another in time (succession of ontogeneses) and in space, and which have the characteristic feature of being able to transmit genetic information.

In regard to nonliving organic matter, under conditions prevailing on Earth it is represented on the one hand by products of the disintegration and transformation of living matter (humus, peat, coal, etc) and on the other hand by accumulations of petrolite type organic matter (petroleum, gas and bitumins), in whose origin abiogenic processes play a determinate role. The interrelationship of problems of the origin of life and the origin of petrolites has been acknowledged by many researchers who had studied the problems of genesis of petroleum. However, proponents of the inorganic theory view the origin of living organisms as a consequence of biochemical evolution of abiogenic petroleum type organic substances (Oparin, 1966; Kropotkin, 1959; Ginzburg-Karagicheva, 1947; Kudryavtsev, 1955), while proponents of the organic theory of the origin of petroleum argue that living organisms were the starting substance for the formation of petroleum hydrocarbons.

The origin of organic compounds which are basic to the introduction of primary forms of life are connected to processes of the synthesis of complex prebiological systems in the atmosphere and hydrosphere of the Earth according to a number of investigators (Oparin, 1966; Shramm, 1966), while certain researchers attribute the origin of living matter to the emersion of juvenile waters (hydrotherms), which carry abiogenic organic compounds to the surface (Florovskaya, 1964).

In the opinion of P. N. Kropotkin (1959), "...by way of elimination it is easy to arrive at the conclusion that the only one of the organic substances spread about in the Earth's crust which might be viewed as the source of the primary forms of life is petroleum or complex hydrocarbons which are close to it in composition."[6] In other words the connection between "discrete" and "indiscrete" strains of evolution does exist but it has a nature opposite to that which the proponents of the organic theory attribute to it (Pozharitskaya and Starostin, 1970).

In recent years a number of investigations of the composition and structure of organic matter of extraterrestrial origin have been conducted. Results indicate a widespread occurrence of organic compounds in cosmic space: in the composition of ice kernels of comets, in the atmosphere of cold planets and, /272
above all, in meteorites (Levin, 1966; Miller, 1966). In conformity with modern cosmogonic concepts we have every reason to suppose that organic compounds of cosmic origin are the most likely source of matter for subsequent evolution of complex organic substances in the development of planetary systems.

The aim of the present work is an attempt at reconstructing certain characteristic peculiarities of this starting mixture of organic substances, which we shall call a protoorganic complex, in connection with the future possibilities of setting up experiments for discovering extraterrestrial forms of a protoorganic complex and the results of its evolution.

The simplest form of the occurrence of organic compounds in cosmic space is hydrocarbon and hydrocarbon radicals in the cores of comets: C_2, C_3, NH, NH_2, CH, CN, CH_2, C_2H_2, C_2H_4, CH_4 (Levin, 1966; Miller, 1966; Vodvykin, 1967). More complex organic compounds were discovered in a certain type of stoney meteorites, carbonaceous chondrites. The amount of organic matter in them was 6-7% (Cohen, 1894).

The complex organic matter of meteorites is represented chiefly by high-molecular polymers (Vdovykin, 1967). In addition we established the presence of bitumen-like matter (up to 0.4%) in the composition of carbonaceous chondrites

[6]See [18], p. 88.

(Nagy, 1968). This substance consists of a complex mixture of aromatic hydrocarbons (up to 60%), saturated by a hydrocarbon with predominance of structures with an even number of carbon atoms (Oro et al., 1965). Isoprenoid hydrocarbons are also included among the saturated hydrocarbons which were discovered (Oro et al., 1967).

Fatty and aromatic acids, purines, pyridines and certain amino acids were discovered to be among the organic compounds in some carbonaceous meteorites (Vdovykin, 1967). According to investigations of many scientists, the formation of organic compounds of meteorites is related to abiogenic synthesis from the simplest gaseous compounds of carbon, hydrogen and nitrogen. The majority of investigators suppose that synthesis occurred under conditions of agglomeration of small asteroidal bodies, fragments of which became meteorites.

The conditions for agglomeration of the asteroid bodies are close to the conditions for the formation of protoplanetary concentrations. The organic compounds which originate at this time are related to the early or protoplanetary age of evolution of the protoorganic material which is characterized by the formation of high molecular compounds which have a predominantly hydrocarbon composition.

Peculiarities of the evolution of the protoorganic material under planet conditions are similar to those in the geochemical evolution of the Earth. In /273
our opinion, the formation of petrolytes and the biosphere is related to two parallel lines of development of a single protoorganic material, whose specific properties are determined by different ecological, geochemical and biochemical conditions[7]. Hence, there are certain features of the similarity and difference in composition and structural peculiarities of the organic compounds which are related to the systems. Their general peculiarities which reflect the evolutionary connection to the protoorganic material are: starting composition and distribution of microelements, presence of similar tyrole compounds (pigment), asymmetry of compounds and cross-linking elements, and also a similarity of isotope composition. It is mainly these features, as will be clear in the

[7]P. N. Kropotkin (1959) and N. N. Dzhaparidze (1959) adhere to a similar point of view on the origin of life and petroleum.

following discussion, that are presented for analysis of the condition and level of evolution of the organic matter under extraterrestrial conditions.

Composition of the microelements and the extent of their dissemination in living matter and in the composition of petroleum are similar according to the data of Vernadskiy (1954) and Vinogradov (1963). The characteristic microelements of petroleum: calcium, iron, magnesium, potassium, sodium, iodine, bromine, zinc, phosphorus and vanadium are in living protoplasm.

In order to discover the level of evolution of organic matter, however, it is not essential to know the elemental composition of the sample so much as it is to find the presence of characteristic complex compounds, in particular porphyrin pigments. The prevalence of white uniform pigments (porphyrin, chlorophylls, hemoglobin) in the composition of all systems, which we related genetically to the protoorganic material, indicates that dye substances which contain pyrrole rings were included very early in primitive photosynthetic reactions. Primary pigmentation occurred due to protoporphyrins, which were synthesized from ammonia and acetylene (Dzhaparidze, 1959) with the participation of vanadium and nickel, which during a further evolution were replaced by magnesium in chlorophyll and by iron in hemoglobin (in other respects the two latter pigments preserved the chemical structure of the ancestral protoporphyrins in their prosthetic groups — four pyrrole rings, a complex bond of an atom of the metal with tertiary atoms of rings 3 and 4, methyl and vinyl groups in the β-positions). The prophyrins were retained in a relict, unsubstituted form in petroleum and in some animal organisms (coporphyrins in eggshell, conchporphyrin in mollusk shells). Such substances as magnesium — protoporphyrin which is found in chlorella, or hematoporphyrin may serve as intermediate products which are transitional to typical pigments.

The formation of primitive pigments through time is related to the conditions under which the protoorganic material was formed (Hoggeson, Beker, 1964; Florovskaya et al., 1968). Therefore other planetary samples must be investigated in the presence of these pigments. /274

Naturally the protoorganic photosynthesis was not connected to the highly organized organelles such as chloroplasts; however, the transitional stages towards the latter from accumulations of protoporphyrin molecules may be traced beginning with glycine and succinate, elementary components of heme and chlorophyll which existed at this early stage (Hoggeson, Beker, 1964). Magnesium was included in the protoporphyrins in the following phase as was iron (the trivalent variety) due to the action of oxidative catalysts. Iron and magnesium continued to play the role of catalysts, the first in hemic dissimilation, and the second in chlorophyll assimilation.

A feature even more important than pigmentation for analysis of other planetary organic matter is the presence of optically active compounds. Under conditions on Earth it is characteristic both for petrolytes and for living matter. Up until now the optical activity of petroleum has been put forward as evidence favoring the organic hypothesis since this property is characteristic for biogenic substances; in the latter, in contrast to the majority of petroleum elements, levorotation predominates which forces us to introduce a number of additional assumptions into the hypothesis since the transition from levorotation to dextrorotation lends itself to explanation no easier than the origin of the optical activity de novo.

A way out of this seeming contradiction was proposed by D. V. Belikhov, according to whom "...the optical activity of original petroleum may have an origin completely independent of that of organisms, whose rudimentary optically active properties were already acquired by the primary organic compounds before the appearance of the first organisms."[8] We also think that the optical activity is a very ancient property and possibly arose by the time of the protoorganic material, although not by the earliest stages of its development.

The early (preplanetary) stages of the development of the protoorganic complex were evidently characterized by synthesis of racemic mixtures. The fact that numerous attempts at discovering the optical activity of solutions of organic matter of meteorites were in vain (Vdovykin, 1967) is confirmation of

[8] *Uchenyye Zapiski Kazanskogo Gos. Ped. in-ta, Fak-t Yestestv.*, p. 184, 1950.

this. The origin of optical activity dates from the planetary geochemical stage of evolution of oranic compounds which are included in the composition of the protoorganic complex. Hence, it follows that study of the optical activity /275 of samples of organic matter of extraterrestrial origin is of paramount importance for discovery of life on other planets.

We may suppose that the division of racemic mixtures of the protoorganic complex was related to processes of initial differentiation of the protoplanet and continued in later stages of the geochemical evolution resulting in the formation of the ancient atmosphere, hydrosphere and the beginning of the formation of the Earth's crust. The formation of protopetroleum systems rich in hydrocarbon occurred under deep regenerating conditions at high temperatures and pressures. The most mobile hydrocarbon system was carried to the surface along with the hydrothermal and pneumatolytic fluids. According to earlier data (Zezin et al., 1967) the end products in the process of hydrothermal differentiation of abiogenic hydrocarbons were enriched by a light carbon isotope (C^{12}). The light isotope composition of carbon of the abiogenic hydrocarbon and, above all, the petroleum-type highly molecular substances related to the process of magmatic differentiation of deep matter in the Earth, was inherited by the protobiological line of development of organic matter. This was also confirmed by the light isotopic composition of the carbon of bitumen-like matter of meteorites (Vdovykin, 1967), and also the carbon of graphite from precambrian schists (Zezin et al., 1967), which are evidently either products of complete transformation of the most ancient organisms of the Earth or are products of the destruction of precipitated highly molecular components of a protoorganic complex.

Thus, the light isotropic composition of the carbon of living matter, petroleum and bitumen-like matter of meteorites is one of the most important genetic features which indicates their connection with the processes of the evolution of a single starting mixture of organic substances. We can suppose that there was also a division of the protoorganic racemic mixtures parallel to the isotope division, that is, the petroleum system of deep zones of the Earth's crust was enriched by dextrorotatory optically active substances, and the probiotic organic matter which came to the surface of the Earth

was enriched by levorotatory active substances. The mechanism for assimilation by biological systems of the light isotope of carbon and the levorotatory active substances was retained during the process of later evolution of the biosphere and became one of the determining features of living matter. The microbiological conversion of deep accumulations of petroleum and gas accompanying the appearance of living organisms promoted the generation and enrichment of optically active components in petroleum elements and the formation of biogenic gases (methane) with the light isotopic composition of carbon. Correspondingly, enrichment of specimens by the light isotopes of carbon may serve as an indication of their biotic favoring nature. /276

A similar possibility may not be excluded in relation to the presence of a gas polymer. Polymer compounds are characteristic both for the meteorites of petrolytes and for living protoplasm. According to the point of view of Dzh. Bernal, and supported by A. I. Oparin, "...during an increase in temperature in the formation zone of Earth-type planets the surface, glacial and condensed layers evaporated and volatilized, but more or less highly molecular organic substances were preserved thus making the beginning for the formation of those carbon complexes which can now be discovered in carbon-like meteorites" (1966, p. 68).

Hence, the importance of polymerization for probiotic and protopetroleum systems in their struggle "for existence" is evident; without polymerization the components would have volatilized into space like helium or hydrogen as evidently happened with significant masses of the protoorganic material and other gases.

After their introduction the protopetroleum and probiotic systems could not immediately completely separate from their protoorganic material. Their chemically active components continued to interact with it and with the surrounding environment which was partially expressed in their hydrogenation. It is natural to suppose that some of the most important factors which determined the further development of the protoorganic complex were the processes of polymerization and condensation which went on in various ways in the probiotic and protopetroleum systems.

In the carbonaceous matter of the meteorites carbon is not amorphous, but has carbon chain macromolecules ...–C–C–C–... (Vdovykin, 1967). Dzh. Oro compares their structure to the structure of melanin and other primitive biogenic substrates, placing both under the conception of an "irregular polymer"[9]. Fatty acids with up to 30 carbon atoms were found in a molecule in an Orgian chondrite, while C_{16-18}, C_{22} and C_{27} are the most widespread. Data on the polymerization of hydrocarbons in petrolytes is also known which may possibly have definite primitive features in common with the polymerization which took place at early stages in the development of the protoorganic complex (preplanetary).

The formation of molecules of organic compounds in probiotic systems led to an increasing accumulation of chemical energy. Micromolecules rich in chemical energy, characterized by a high reaction capability, inherited a /277
preference for their own "survival" and in the end only they turned out to be promising for the evolution of highly molecular organic matter.

Chain polymers are also encountered in organisms (rubber, silk fibroin, and cellulose), but, in contrast to the protopetroleum lines of evolution, helical (fibrillar proteins, DNA) and globular polymers predominated in them. Particles of chain biopolymers have a dimension on the order of 5 × 10 × 100 Å with a molecular weight of $10^3 - 10^5$. The corresponding indices for petrolytes are significantly lower; it is unlikely that this was any different during the early stages of their development.

The distinction of biopolymerization from polymerization in protopetroleum systems from the very beginning was both in the course of the process of polymerization itself (formation of spatial polymers) and in the material which was attracted to this process for the biopolymers, formaldehyde and ammonia molecules and radicals and hydrogen cyanide, etc. Preprotein compounds, according to Sh. A. Akabori (1966), were condensed from formaldehyde, ammonia and hydrogen cyanide through the stage of aminoacetonitrile:

$$CH_2O + NH_3 + NCN \longrightarrow NH_2 - CH_2 - CN + H_2O$$

[9] See [36].

with subsequent polymerization of the aminoacetonitrile by hydrolysis to polyglycine and ammonia:

$$x\,(NH_2-CH_2-CN)\longrightarrow -(-NH-CH_2-C-)x \xrightarrow{H_2O} \longrightarrow (-NH-CH_2-CO-)-x + NH_3$$

and by introduction of the side chains to polyglycine by means of interaction with the aldehydes or also with the unsaturated hydrocarbon. At the present time this arrangement seems highly likely although we still are not able to concretely determine many details of the process. It would be logical to suppose that melanin-type irregular polymers arose at first, whose synthesis has been accomplished experimentally by ultraviolet irradiation of aromatic amino acids (Blua, 1966). Orderly and more stable types of polymers arose later with the appearance of a structure often designated as "aperiodic crystal." Micromolecules of carbonaceous meteorites and solid bitumens of the petroleum series (anthraxolites, kerites, etc), on the other hand are arranged in chains and have a tendency for transition to a normal periodic structure.

Purine (for example, adenine $C_5H_5N_5$ of five molecules of HCN) and pyrimidine bases also arose by means of polymerization. Worner (1969) studied the reaction of polyadenylic and polyuridilic acids, which occurs with simple mixing at room temperature. The viscosity of the mixture sharply increases as spectrometric shifts occur. The hydrogen bonds which form are of paramount /278
importance for the structure of DNA and its predecessors (Haldane, 1966; Sagan, 1966; Kharada and Foks, 1966).

In spite of the similar features of the two lines in the evolution of organic matter of the Earth's crust which are determined by the relation to the protoorganic material the line of evolution which is the most promising for the development of life is the one bound to the surface of the Earth's crust and having its habitat at the boundary of interaction of the three geospheres: the atmosphere, hydrosphere and lithosphere (Vernadskiy, 1954).

The multiplicity of factors which determine the ecological conditions of the origin and development of probiotic systems promoted the appearance of qualitatively new structural-functional forms the specifics of which must

be understood in order to successfully solve the problem of detecting life beyond the Earth.

Indeed, all biological functions can be carried out only by structuralized formations. This is also true for the microscopic and ultramicroscopic levels as well as for the macroscopic level. Only biological systems and subsystems can carry "functions" in the proper sense of this word, therefore this specific structuralization is one of the most important features by which we can pass judgement on the biogenic origin of a specimen. Living organisms preserved and develop those rudiments of structuralization which were already present at the stage of the protoorganic set while these rudiments did not develop further in the indiscrete line of evolution (petrolites).

Structuralization is characteristic for biotic-favoring systems from the very earliest stages of their development. During a spark discharge in a mixture of methane, ammonia, hydrogen and water by the perfected method of Miller-Yuri with the production of amino acids and peptides (through the stage of hydrogen cyanide and aldehydes) after approximately 48 hours characteristic dense structures (microspheres) with a size of 50 to 100 Å are formed, whose organic matter consists primarily of amino acids, proteinoids and compounds related to them (Grasenbakher and Nayt, 1966). Similar formations are produced during the cooling of a proteinoid solution from 25 to 0° or by means of boiling proteinoid in water with subsequent cooling. "Budding chains" of microspheres, and also blastula-like forms are created while measuring the pH, pressure and other factors. The so-called "Goldacre bubbles" which are formed from the biogenic or abiogenic protein-lipoid film (Oparin, 1966) are adjacent to the blastula-like forms. The coacervate drops obtained by G. Bugenbergde-Yongom in the 1930s from gelatin and gum arabic are a quite characteristic example of the introduction of primary structuralization in a colloid solution. The drops separated out from the surrounding solution are the /279
abrupt contact area and the concentration of the polymers within them reached percentage figures in tens when the percentage in the external solution was in parts per thousand.

One should also note the discovery by M. Kal'vin of "quantosomes" in the chloroplasts of plants which measure about 200 Å and are composed of 200--300 molecules of chlorophyll bound to proteins. The processes which might lead to the synthesis of such elementary structures on a base of amino acid--porphyrin polymers were observed under natural conditions by V. N. Florovskaya (1964, 1968).

At the present time it is difficult to say what kind of shape the probiotic structures had and whether they were uniform. The fact that there are many variants from which these structures may have come complicates the situation. We have noted that the variety of artificially created "protobionts" have a spherical shape and a size of up to 1-2 μ, while the preceding primary "organized elements," found in carbon-like chondrites, have a size of 4-30 μ and not only a circular but also a cylindrical or hexagonal shape (Zelinskiy, 1936; Vdovykin, 1967).

The functioning of two primary organized elements, whatever they were, was possible only after a certain amount of development of the catalytic apparatus. The first catalysts were already present in the protoorganic material or its surrounding environment of the salt of heavy metals or were central organic compounds such as methylamine.

In conclusion we can say that the study of the results of the evolution of organic matter on Earth is essential both for construction of a general picture of bioporesis and in relation to the problem of discovery of life on other planets. Organic matter is distributed in the universe more estensively than life for which the protoorganic complex is a transitional stage. In turn the evolution of organic matter is closely connected to specific conditions of the development of products.

Peculiarities of the planetary evolution of Mars are closer to those of Earth (Lyubarskiy, 1962; Vokuler, 1965).

Proceeding from our concepts of the coexistence of a set of protoorganic compounds in the protoplanetary stage we have every reason to suppose that similar peculiarities of planetary evolution will also result in similar forms of organic life. And although the specific conditions for development of a

biosphere may differ from those of the Earth the general features which reveal the connection with a protoorganic complex, the characteristic composition of microelements, optical activity, the isotope composition, and signs of structuralization must be retained.

Searches for life on other planets must envisage discovery of both the common properties of a set of organic compounds and the functional-structural /280
peculiarities which are characteristic for the biological line of evolution of organic matter. An adequate methodology for detection of extraterrestrial life must be guided by the necessity of dealing with organic matter which has become more complex at quite different stages of the development of protoorganic complexes. Thus, the methodology for discovering extraterrestrial life or the level of evolution of organic matter must consider the elemental and isotopic composition of organic compounds, the presence of pigments and polymers and optically active molecules in them and the nature of the functional structuralization.

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EFFECT ON INFUSORIA OF PHYSICAL CONDITIONS WHICH SIMULATE THE ENVIRONMENT ON THE SURFACE OF THE PLANET MARS

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ABSTRACT: Substantiation is given for the use of protozoa in particular infusoria, in exobiological experimental investigations. The authors show the high lability of unicellular animals and their adaptability to adapt to extremal conditions of the external environment, in particular to conditions existing on the planet Mars. The infusoria *Colpoda maupasi* are able to multiply at low atmospheric pressure (10-5 mm Hg) in a gas environment consisting of nitrogen, in an atmosphere of carbon dioxide at 15-16 mm Hg and less than 1% oxygen. The tests were conducted under conditions that sealed ampoules in anaerostats and in a "photostat" with a circulating atmosphere. The infusoria *C. maupasi* survived as dormant cysts, when placed into the "photostat" in moistened sand at the trophont stage with daily temperature changes from +25 to -30° (up to two weeks), in an atmosphere of carbon dioxide CO_2 with a pressure of 10 mm Hg and during eight hours of daily ultraviolet irradiation and in visible light.

Daily changing temperatures above 0° increased the division rate of the infusoria in comparison to constant temperature conditions even though the sums of the temperatures were identical. The data obtained allowed us to conclude that the existence of not only dormant but also active forms of protozoan animals which have adjusted to life under varying temperatures and which have a short life-cycle is possible under certain conditions on the planet Mars.

Exobiological investigations conducted with microorganisms (bacteria, /281
yeast) show that they are able to withstand conditions which differ significantly from those of Earth. Conditions to which organisms did not adapt during their evolution we decided to extremal (Lozina-Lozinskiy, 1963). On the basis of our own data and data in the literature it seemed to us that among the kind of Protozoa there are unicellular animals which have adjusted excellently to severe conditions of certain biotopes of the Earth and are resistant to extremal actions.

From this point of view Protozoa belong to those organisms whose study is of great interest in connection with problems of exobiology. Wide distribution and adaptation to life in biotopes with significant fluctuations of humidity and temperature are characteristic for many phyla of protozoa; thanks to the /28 formation of dormant cysts they can withstand arid conditions, superlow temperatures, vacuums, and may be disseminated great distances by air currents. Thus, on Earth the protozoa occupy different ecological niches.

It can be confirmed that Protozoa are characterized by a greater plasticity in individual adaptation to surrounding conditions than bacteria. Such adaptive reactions as "prolonged modifications," that is, rapidly occurring adaptive reaction to external action without change of the genotypical properties, are typical for them. The plasticity of functions of Protozoa, especially for infusoria, determines the complexity of their behavior which resembles the nature of the behavior of animals which have a central nervous system, which increases their ability to adapt to the surrounding environment.

The principal importance of studying the resistance of Protozoa, to extremal conditions consists in the fact that if they are capable of adapting to it and can withstand conditions similar to those which take place on Mars, we cannot eliminate the possibility of there being life on this planet.

We investigated the resistance of certain infusoria to a number of different extremal actions in their total effect and, in particular, to those conditions which organisms experience during simulation of conditions on the planet Mars. The reactions to extreme conditions were studied mainly in the soil infusoria *Colpoda maupasi*.

This article discusses data on the survival rate and reproduction of these infusoria during the action of such factors as varying density of the atmosphere of the air, nitrogen and carbon dioxide on them; the possibility of its existence with a small amount of free oxygen in the environment and in an atmosphere saturated by carbon dioxide. The action of a gas medium and organisms in the active state is inseparably connected to the temperature of the medium and its fluctuations, therefore we included certain results of tests with varying atmosphere in this article.

Density of the atmosphere. The survival rate of microorganisms, of protozoa and cells of multicellular animals does not change when there are very wide limits of atmospheric pressure.

It has been established that spores, pollen of flowering plants, seeds of different plants in the dry state can withstand a high vacuum (10^{-5}-10^{-6} mm Hg) (Becquerel, 1925, 1936; Shmidt, 1955), but bacteria in the normal state can withstand a superhigh vacuum (3.6 10^{-10} mm Hg) (Imshenetskiy, 1962; Portner et al., 1961). Significant rarefactions of the atmosphere (about 5.0-0.1 mm Hg) have no effect on dried microscopic animals — rotifers, nematodes, Tardigrada and cysts of Protozoa (Brand, 1951; Bychenkova, 1966; Kitching, 1939; Rahm, 1926; Taylor et al., 1936). But in a number of cases a high vacuum causes damage to cells and reduces their capability of life (Imshinetsky, 1963; /283
Imshinetsky et al., 1965).

The problem as to which degree of rarefaction of the atmosphere allows organisms to be active and reproduce has been studied a great deal less (Vallentyne, 1965). Lower atmospheric pressure may have the following action on biological objects: 1) cause boiling of body fluids at temperatures significantly lower than 100°; 2) increase evaporation of water and dry out cells; 3) causes sublimation in the case of freezing of a system which also brings about desiccation; 4) lowers partial oxygen pressure which leads to disruption of respiration and altered metabolism. Obviously, other reactions may exist, caused by a drop in atmospheric pressure, which are not known in detail.

Tests of the reaction of active infusoria to reduction of atmospheric pressure were conducted in hermetically sealed vessels in anaerostat tests with a volume of 2:5 *l*, in sealed ampoules of 3 and 15 m*l*, and in a "photostat" low-pressure chamber, through which air, nitrogen or carbon dioxide at a given reduced pressure was passed at a rate of 150 m*l* per minute. The experiments showed that at air pressure within limits from 65 to 5 mm Hg infusoria *Colpoda maupasi* in the active state at stages of trophonts and cysts can withstand minimal pressure and reproduce at the same rate as the control infusoria under conditions of periodic conditioning of the atmosphere. At the same time we

found that the infusoria in sealed vessels perish during minimum pressure and cease to reproduce during rarefaction up to 10 mm Hg. The reason for these differences is connected to the fact that conditions of hypoxia and anoxia occur in sealed vessels due to the binding of oxygen cells. Therefore reduction or cessation of reproduction occurs in a closed system in an atmosphere of nitrogen and carbon dioxide which contains less than 1% oxygen even at a pressure of about 15 mm Hg. We were not able to determine the limit at which rarefaction causes death to active cells because the further change in the density was related not only to hypoxia but also to desiccation of the medium and transition to the encystment state.

However it was possible to explain the specific action of a relative vacuum by placing the infusoria in a rarefied atmosphere of pure oxygen.

Boiling of water at the density of the Martian atmosphere of about 20 mb occurs at +18° and at 5.58 mm Hg with a temperature of 0°, but the process of boiling is evidently not present in the cell. Thus, the infusoria *Colpoda maupasi* and *Paramecium caudatum* may live and reproduce at such pressures for an indefinitely long time. Boiling of the liquid ceases rather quickly as a result of the establishment of equilibrium of gases in a liquid medium and /284 atmosphere.

Action of an atmosphere of varying composition on infusoria. We tested an atmosphere of nitrogen which contained 0.5% oxygen (technical nitrogen) and 0.0005-0.0002% oxygen (chemically pure nitrogen), and also an atmosphere of carbon dioxide which contained impurities of other gases amounting to about 1.5% (nutritive carbon dioxide)[10]. Composition of these impurities is unknown, but judging from the fact that the aerobic infusoria reproduce in an atmosphere of carbon dioxide, there must have been oxygen in it. In an atmosphere of these gases we conducted experiments at atmospheric pressures of 760, 60, 30, 15, 10 and 5 mm Hg (limit of variations was ±2 mm Hg).

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10 A small number of tests were conducted with welding carbon dioxide which contains 0.5% impurities of other gases.

Atmosphere of nitrogen and oxygen. According to recent data, the principal gas of the atmosphere of the planet Mars is nitrogen. As a result of analysis of data obtained from Mariner IV (Kliore et al., 1965), recent astronomical data (Moroz, 1967; Owuen et al., 1968) it was established that the main component part of the atmosphere of Mars is carbon dioxide; oxygen, also in recent investigations, was not directly detected.

Therefore, from the point of view of studying conditions of the existence of organisms on the planet Mars, simulation of a nitrogen atmosphere is not justified, but previously published tests (Bychenkova et al., 1970) do lose their importance because the oxygen requirement of the aerobic Protozoa in a system of an inert gas was clarified. Tests in an stmosphere of nitrogen containing about 0.5% oxygen showed that at an atmospheric pressure of 15 mm Hg and higher the infusoria *Colpoda maupasi* grow at room temperature at the same rate as the control Protozoa in a normal atmosphere of air.

Reproduction of infusoria under conditions of a changing atmosphere in the "photostat" chamber occurs at a pressure of 5-10 mm Hg of pure nitrogen which contains only 0.0005-0.0002% O_2, or in other words with an amount of oxygen 2-4 million times less than the amount which exists in the Earth's atmosphere at sea level. Before these tests were conducted it was known that many infusoria are capable of anoxybiosis with transition to the glycolytic type of metabolism, but it was not determined whether they can reproduce under these conditions.

Atmosphere of carbon dioxide. It is known that invertebrate animals can withstand a concentration of carbon dioxide hundreds of times greater than homeothermic vertebrates, that the problem of the existence of cells of animals and unicellular heterotrophic organisms in an atmosphere of pure carbon dioxide or one containing traces of other gases has not been investigated (Beadle, 1961; Hawrylewicz, 1967). Tests with *Colpoda maupasi* were also conducted in an atmosphere of nitrogen: a) in an anaerostat and ampoules and b) in a low-pressure "photostat" chamber, in which the gas medium was renewed (Figure 1). In the first vessel the atmosphere was not changed during the test and its composition obviously changed due to the effect of the vital

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activity of the infusoria, and also due to the activity of bacteria (*Bacillus subtilis*), which were in the medium as a nutrition source. In the "photostat" the carbon dioxide was continuously pumped through the low-pressure chamber at a rate of 150 ml/min, and its composition remained constant. In tests in the anaerostat with a volume of 2.5 l a definite amount of the infusoria were placed into holes of microaquaria. In other tests a precise number of infusoria were placed into ampoules with a volume of 3 and 15 ml, from which the air was first withdrawn to a pressure of 3 ml Hg; then it was filled with carbon dioxide to the necessary density after which the ampoule was sealed. The Lozina-Lozinskiy salt medium was used as the medium (1948).

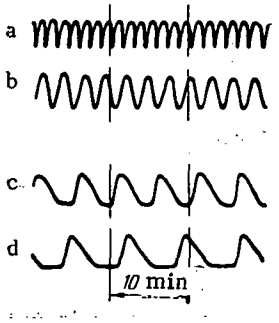

Figure 1. Diagrams for Pulsating Pumping of a Gas Through a Low Pressure Chamber for Ten Minutes. a, 8 times; b, 4 times; c, 2 times; d, once.

We placed either five or twenty infusoria into the ampoules Below are given the average data from seven tests in which infusoria at the stage of trophonts with five species in each ampoule having a volume of 15 ml were kept for four days under varying pressures of carbon dioxide. The average number of infusoria by the end of the gas pressure test was as follows:

Pressure, mm Hg	5	10	15	30	60	760
Number	7.7 ± 20	380 ± 130	1,430 ± 46	560 ± 16	480 ± 50	0

The greatest number of infusoria were detected at a pressure of 15 to 60 mm Hg, and their rate of reproduction was the same as for the infusoria at normal air pressure.

With a pressure of carbon dioxide equal to 1 atm, infusoria quickly perish after fifteen minutes in a number of tests; with pressures from 60 to 5-3 mm Hg active infusoria were observed for fourteen days. Similar results were obtained in ampoules having a volume of 3 ml.

One hundred strains of active infusoria (trophonts) and fifty dormant /286
cysts were subjected simultaneously to the action of CO_2 in an anaerostat. The maximum rate of reproduction under these conditions was also observed at a pressure of 15-30 mm Hg. Retardation of the rate of division was observed only at a CO_2 concentration (with a 1.5% admixture of other gases) which exceeded the concentration in the Earth's atmosphere by 300 times. Under conditions of an atmosphere of carbon dioxide at reduced pressures the infusoria are capable of emerging from the cysts. If the CO_2 atmospheric pressure is reduced to 10 and 5-3 mm Hg, then the condition of the infusoria worsens, possibly due to hypoxia and an increase in sensitivity to O_2 deficiency in the presence of carbon dioxide.

The resistance of infusoria to the CO_2 atmosphere which circulates through the low-pressure chamber was studied in combination with other factors of the Martian environment.

Action of the combination of factors. We created the following conditions in the "photostat" chamber. Carbon dioxide (containing about 1.5% admixture of other gases) passed through the chamber containing the organisms at a pressure of 5 to 10 mm Hg (in different experiments) throughout the entire test. The daily progress of the temperature is shown in Figure 2. The minimum temperature "at night" was -25° or -30°, the maximum "during the day" was +25°. Daytime illumination was simulated by a DKSSh-1000 xenon lamp, whose spectrum is close to sunlight (Borisov and Gavanin, 1966; Nilender and Gavanin, 1968). The length of day was 10-12 hours with light intensity of 50,000 lux. Throughout this time the total amount of ultraviolet energy irradiated by the lamp to the medium with the objects reached 2,240 $\mu W/cm^2$

A definite number of infusoria in the active stage or in cysts was placed with a Lozina-Lozinskiy medium into test tubes or Petri dishes, in which there was pure quartz sand. Moistening of the sand with a Lozina-Lozinskiy salt /287
solution was always excessive at the beginning of the test. We measured the amount of evaporated water (the Petri dishes and test tubes remained open during the test in the working chamber of the "photostat") for weight loss at the end of the test. At the completion of the test in the "photostat" each sample

because of the desiccation, was again moistened by the Lozina-Lozinskiy medium and we determined the number of active excysted infusoria in it by the culturing method after keeping it at a temperature of 22° for 24 hours. Infusoria which were grown outside the working chamber of the "photostat" under normal atmospheric conditions and temperature of 22° were used as a control; their rate of division was computed.

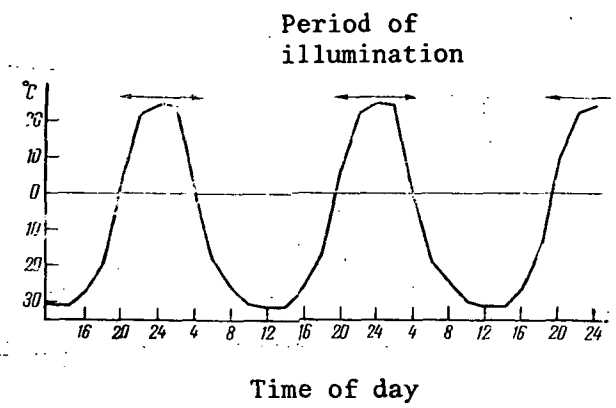

Figure 2. Change in Temperature Inside a Large Pressure Chamber, by Days.

The infusoria in the low pressure "photostat" chamber were subjected to the influence of 1, 2, 4, 5, 13 and 14 day temperature and radiation cycles, throughout which they experienced freezing, desiccation and irradiation in an atmosphere of CO_2. Evidently the irradiation did not have any great significance because the infusoria, being shielded by particles of sand, were not subjected to the direct action of ultraviolet rays.

We have presented data on the survival rate of the infusoria in the table. After 13-14 cycles, three to thirty-five percent of the initial active infusoria which were inserted in the sand remained alive.

On the basis of specially conducted tests in the "photostats," in which we studied the effect of change of temperature and rate of desiccation to encystment and excystment of the infusoria and on the survival rate of active forms under normal pressures and composition of the atmosphere, we were able to arrive at the following conclusions.

For an encystment of the *Colpoda maupasi* it is necessary that the temperature of the medium be above 8°, so that during a quick drop in temperature /288
the part of the infusoria which has not been prepared for the formation of dormant cysts perished as a result of freezing during the "Martian" night.

In case of a rapid desiccation of the medium the formation of cysts would reduce the percentage of deaths from cooling and freezing, but for encystment the rate of desiccation of the environment should not proceed rapidly. The most optimum rate is a desiccation duration of about six hours. Therefore, terrestrial organisms under the conditions on the planet must adapt themselves to managing to emerge from the cysts during the period of warm daylight hours, reproducing and forming dormant cysts before cooling and freezing of the medium or before its vigorous and rapid desiccation. From this it also follows that an excess of humidity is an unfavorable factor during abrupt changes of temperature (falling below 0°).

TABLE. SURVIVAL RATE OF INFUSORIA *COLPODA MAUPASI* IN THE "PHOTOSTAT-1" DURING THE COMPLEX ACTION OF PHYSICAL FACTORS.

Daily cycles	Number of tests	Number of tests, in which they did not survive	Number of active infusoria in the test	Percent of starting number which survived
1—2	6	2	100 1500 3000	0,3 1,3; 1,3 2,5
4—5	39	22	100 320—500 600—800 2000—5000	8,8; 6,0 11,0; 39,0; 13,0 15,0; 0,8; 0,7; 28,0; 25,0; 1,0 5,0; 10,0; 0,2; 0,25; 0,3; 10,0
13—14	12	6	140 500 2000	35,0; 0,8; 1,6 3,0 1,25; 2,5

Note: Commas indicate decimal points.

If fine quartz sand which has a small capacity for moisture is taken as a model for a solid medium then the wetness (by weight) from 10 to 1.5% ensures an active existence not only for amoebae and flagellates, but also for infusoria (Lozina-Lozinskiy, 1930). In the test described above moisture evaporated from the sand after 2-4 daily cycles in the "photostat" chamber which caused cessation of reproduction. Unfortunately under the conditions in these tests in the "photostat" we were not able to maintain any desirable conditions of moisture.

Variable Temperature. Enormous daily fluctuations of temperature are noticeable on the surface of the planet Mars, which differ at various latitudes and at various seasons. According to the data of different authors the highest temperatures in the days are at the Equator: +25-30° and from -30 to -80, -90° at night. It should be remembered that these fluctuations become less as one goes deeper into the soil or ground. Therefore in order to predict the possibility of the existence of terrestrial protozoan organisms on Mars one must explain how they will react to daily changes of temperature and to supercooling.

As a rule dormant cysts of Protozoa withstand supercooling when active stages usually perish during freezing of the medium and intracellular crystallization. Among the active forms only the parasitic species survive ~~copius~~ cooling (trypanosomes, malarial plasmodia and others) and they do so mainly in the presence of protective substances. As was shown by V. N. Bychenkova (1966) dormant cysts of *Colpoda maupasi* withstand freezing in a liquid medium down to -196° and down to -79° with preliminary cooling. Consequently, the low temperatures observed on the surface of Mars do not present any danger for dormant cysts. However, it remains unknown how the active stages of infusoria will behave under conditions of daily changing temperatures above 0°.

In order to explain the role of changing temperatures above 0° we conducted /289
tests on *Paramecium caudatum*. E. I. Zaar (1966) established that daily changes of temperature within the limits of 28 to 0° act variously on paramecia depending on the range of fluctuations and the maximum and minimum temperatures in the tests.

The average rate of division for six days under constant and varying temperatures is presented in Figure 3. The temperatures rose and fell gradually throughout the day according to a given program in such a way that the sum of the temperatures in the tests was constant, average and variable temperatures were the same. At a temperature of 0° reproduction did not cease but it was quite retarded, being 0.06 of a generation daily. The data showed that under conditions of changing temperatures the rate of division was higher than under constant temperatures; the maximum difference was observed at an average

temperature of 16°. The infusoria reproduce twice as quickly during a change of temperature on the average by 22 to 10° a day (on the average +16°), than at a constant temperature of 16°. In tests in which the daily range of temperature fluctuations was not 12°, but 6°, the effect of variable temperatures became less. If the range of daily fluctuations of temperature was very great, as for example in tests where the temperature varied from 28 to 0°, stimulation of the vital activity was not observed, evidently as a result of the fact that extreme temperatures have a depressing action.

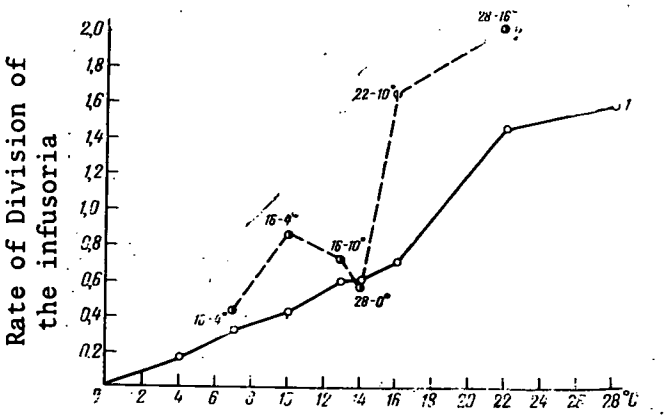

Figure 3. Effect of Variable (2) and Constant (1) Temperatures. *Paramecium caudatum.* Digits on curve 2, limits of fluctuations of temperature for a day.

A change of temperatures from 28 to 0.5° led to the appearance of a certain percent of deformed species; in spite of this fact the paramecia later adapted to such a temperature cycle.

From these data we were able to conclude that significant fluctuations of temperatures, within which limits extreme temperatures do not have an injurious effect, sharply increased the energy of the vital activity, and intensified the rate of reproduction. The fact that cell division synchronizes during daily changes of temperatures and occurs at the most optimum temperatures of the day is of great interest. Thanks to their eurythermy infusoria adapt not only to fluctuations of temperatures but also to reduced temperatures as can be judged from their rate of reproduction (see Figure 3). The eurythermy must encompass all stages of the vital cycle. Thus, in the case of ontogenetic adaptation of the active stages

of *Colpoda maupasi* to reproduction at lower temperature as a result of development at a temperature of about 0°, adaptation which is expressed in the capacity to encyst at this temperature, at least for a short inverval of time, does not occur. Consequently, adaptation to cold of some active forms is not very useful for the species and population.

Humidity. The problem of the role and effect of humidity on organisms /29
under conditions of the Martian environment is especially controversial. On the basis of existing data on the negligible amount of moisture on the surface of Mars and the necessity of water for life the assumption is generally made that the dryness is the chief factor which limits the active existence of organisms on this planet. In actuality, tests show that not only Protozoa but also bacteria need water in quantities which may not always be detected on the surface of Mars (Hawrylewicz et al., 1967). The lack of aquatoria and the desiccation of the environment, which is determined by evaporation of water into the atmosphere, are, it seems, conditions which prevent the development of life. According to the positions favored by some paleontologists (Katterfel'd, Frolov, 1967), the existence of permafrost is possible on Mars and even, though less likely, underground oceans (Davydov, 1960).

As we know from agricultural soil science, the structures of virgin soils not only retain moisture in strong winds, but also absorb it actively from the air. In view of this moisture may accumulate in soil layers both day and night (while dew is falling), and this process may predominate over evaporation of water into the atmosphere which occurs on Earth mainly as a result of evaporation from plants. In other words, given corresponding structures of soils and grounds moisture may be retained in them due to capillary and other forces, in spite of the dryness of the atmosphere. Therefore there are not sufficient grounds to decide on the absence of moisture on Mars based on the small amount /291
of water vapor in its atmosphere.

Thus, we have shown that among Protozoa complexly organized infusoria in a condition of dormant cysts may withstand experimentally created conditions in a laboratory which simulate (to a significant degree) the ones which exist on the planet Mars. Under certain tolerable conditions such as periodic

increase of moisture in soil or earth in such an amount that water becomes physiologically active for a given species, infusoria may reproduce in an atmosphere with a high CO_2 content, which exceeds the amount of CO_2 in the Earth's atmosphere by 100 times, with traces of oxygen and low pressure as observed on Mars. Radiation of the sun evidently does not prevent the development of soil organisms on the surface of Mars, if they are shielded from ultraviolet irradiation by solid particles.

Infusoria in the state of dormant cysts can withstand reduction of temperatures and daily changes in temperature within limits of +25 to -30 and -79° and lower; but in order that reproduction occur under these daily fluctuations of temperature it is necessary that unicellular organisms are able to carry out their daily vital cycle in a shorter period of time than is the case for *C. maupasi*. It is fully possible that forms will be found among the Protozoa which are able to carry out their mytotic cycle during the period of positive temperatures on Mars, and will be able to form dormant cysts and excyst. It is even more likely that such forms would be able to enter into the evolutionary process on the planet Mars.

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PROBLEM OF THE SURVIVAL OF MICROORGANISMS UNDER CONDITIONS SIMULATING THOSE ON MARS

V. M. Rumyantseva, V. L. Levin and M. A. Rybin

ABSTRACT: An aqueous suspension of microorganisms was placed in sand which was enriched by organic substances. Test tubes with sand were placed open into a low pressure chamber of a "photostat" device, where they were kept for 2-14 days in an atmosphere of carbon dioxide at a pressure of 10 mm Hg. The temperature throughout the day changed from +25° to -25°. The most resistant of the museum cultures tested by us were the strains of *Micrococcus luteus*, but among the microorganisms which we separated from Antarctic soil there were two unidentified forms C_1 and A_{14}. Concentration of the cells in these cultures increased during the tests by 1-2 orders.

The action of a set of factors which simulate conditions on the surface of /292
Mars and their effect on microorganisms has been studied for many years in certain laboratories (Levin, 1968). The concepts of these conditions change according to the successes of astrophysics. Data obtained by the U. S. space probe "Mariner-IV" confirm the presence of a lower barometric pressure on Mars than was previously considered and as a consequence, a higher concentration of carbon dioxide. Therefore conclusions from biological experiments made in 1965 require reexamination and refinement.

The purpose of our paper was a study of the possibilities for the survival and development of microorganisms under conditions approximating Martian conditions. Our investigation was carried out on the automatic "photostat" devices (Zaar et al., 1970). This device permits simultaneous investigation of the action of a set of such factors as pressure, gas medium and cyclical change of temperature (in a low pressure chamber) on microorganisms, and also the action of only the cyclical changes of temperatures with normal air atmosphere (in the working chamber).

The temperature in both chambers varied from +25° to -25° (in certain experiments down to -30°). Positive temperatures were maintained for eight hours

a day (3-5 hours at the maximum level); a temperature of -25 to -30° was maintained for 4-5 hours.

The gas medium consisted of 98.5-99% carbon dioxide, which was supplied from cylinders with "welding" and "nutrient" grades of compressed CO_2. The gas circulated through the chamber in a pulsating stream throughout the entire experiment.

The pressure was reduced 30-40 minutes after the beginning of the test to 10 ± 2 mm Hg and was maintained at this level to the end of the experiment.

The duration of the tests was from two to fourteen days.

The objects of the investigation were cultures obtained from a museum of the Institute of Microbiology of the DASKhNIL (including *Bacillus cereus, Bacillus mesentericum, Pseudomonas pyocyanea, Ps. fluorescens, Mycococcus luteus*), and cultures isolated by us from soils of the Antarctic (collections from an Antarctic expedition of 1967)[11].

We chose sand as a substrate for cultivation of the microorganisms. Before the tests we baked it, washed it and added 1% (by weight) of a meat-peptone boullion. After this the sand was dried until it reached an air-dry condition and poured in 10 g amounts into glass tubes 15 × 200 mm in size, closed on one end by a rubber stopper and on the other end by cotton stoppers, which were sterilized for one hour at 1 atm.

Then we put 0.5 ml of an aqueous suspension of the microorganism obtained from a two-day boullion culture into the tube. We immediately contaminated three tubes with one strain: one of them, the control, was kept in the thermostat under normal conditions at 22-24°, the other was placed in the low pressure "photostat" chamber, where the organisms were subjected to the action of the indicated set of cultures, and the third was put in the working chamber, where the temperature varied cyclically in ordinary air. Before placement of the tubes in the "photostat" chambers the cotton stoppers were removed which made possible diffusion of the gases between the sand and the gas medium of the chamber.

[11]For this material we express our thanks to our colleague on the expedition, A. F. Pushkin.

We always put 26 tubes containing sand into the low pressure chamber. The humidity of the sand after its contamination by the microorganisms was 5% (by weight), consequently there was 13 ml of water at the beginning of the test in the chamber with a volume of 18 l. Half of the moisture evaporated from the /294
sand during the first two days. Calculation of the number of cells before the test and at the end of it was carried out by the dilution method by means of inoculation in Petri dishes with MPA on the second and third days of cultivation at 22-24°.

We assumed that reproduction of cells in the sand does not occur before substitution of the air by carbon dioxide, because immediately after placing the cultures into the "photostat" the reduction of temperature begins simultaneously with the generation and accumulation of carbon dioxide.

The set of factors which we used seemed to be unfavorable for the majority of the cultures of microorganisms which we studied. After staying for four days in the "photostat" the number of viable cells of *Ps. fluorescens*, *Ps. pyoceanea*, *Bac. cereus* and *Bac. mesentericum* decreased significantly. The results of the tests varied but we did not observe death of all the cells in any one case. This is confirmation of the greater differences in sensitivity within a single population. Especially great fluctuations in the survival rate were observed in the spore-like organisms. Evidently this is related to fluctuation in the number of spores in the separate test tubes.

Some of the cultures tested were more resistant. Thus, the most resistant of the museum cultures was *Micrococcus luteus*. We conducted twenty-two tests with six strains of this species. After four days stay in the low pressure chamber the number of cells of *M. luteus* increased in fifteen tests by 10-100 times, and in seven tests by 3-5 times (table).

Of the thirty-two cultures tested by us which we isolated from soils of the Antarctic, the most resistant were the cultures C_1 and A_{14} (unidentified). After staying for four days in the low-pressure chamber a number of the viable cells of these cultures increased in comparison to the inoculation by a factor of 1 to 2 (table).

SURVIVAL RATE OF MICROORGANISMS IN "PHOTOSTAT" CHAMBERS (NUMBER OF CELLS PER 10^5 g OF SUBSTRATE).

Culture	Inoculation	Thermostat (control)	Photostat	
			Working chamber	Low pressure chamber
Mycococcus luteus, Strain 12	15	1,216	389	447
Mycococcus luteus, Strain 13	20	1,113	1,060	283
Mococococcus luteus, Strain 14	16	2,270	1,166	194
Antarctic Form C_1	2	330	152	173
Antarctic form C_1	17	240	209	199
Antarctic form A_{14}	34	596	--	486
Antarctic form A_{14}	34	422	--	522
Antarctic form A_{14}	41	--	--	471

Our observations do not contradict the data of other authors concerning the great variety in sensitivity of microorganisms to extremal factors although these tests were set up somewhat differently.

In the Exobiology Department of NASA in the United States of America studies have been conducted on the ability of spore-forming and non spore--forming aerobes and anaerobes to withstand "Martian" cycles of freezing (-70°) and thawing (25°), 4.5 hours a day. It turned out that the vegetative cells of the spore-forming aerobes were less resistant to these conditions than the non spore-forming organisms, although forms were found even among the spore--forming organisms which were capable of growing in "Martian" temperature conditions. The effect of other factors of the Martian environment was not examined (Young et al., 1968).

In a series of studies carried out by the Department of Biology of the Illinois Technological Institute (Chicago), bacteria were placed in a test tube on a felsite-limonite substrate. After washing them seven times with a gas mixture with selective rarefaction the test tube was sealed and subjected to cycles of freezing and thawing.

Gas mixtures which contained 100, 67 or 37% carbon dioxide were used at pressures of 10, 25 and 40 mbars on a background of daily cycles of freezing (to -65° for eight or twenty hours) and heating (up to +25°, respectively for sixteen or four hours). Here also it was shown that various microorganisms do not react identically to these conditions. Thus, spores of *B. cereus*, which were inoculated in the felsite-limonite mixture, did not grow to maturity, but the number of vegetative cells of *Staphylococcus aureus*, even at the most extreme values of the indicated parameters, increased by more than a factor of 10^2 for twenty-eight days (Hawrylewicz et al., 1967, 1968).

Thus, it seems probable that with the development of space biology many forms of microorganisms will be found which are capable not only of surviving but of growing under Martian conditions. Our tests do not enable us to draw conclusions regarding characteristics which determine the survival rate of microorganisms in "Martian" conditions; however, the resistant cultures which we discovered are promising for further investigation.

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THE "PHOTOSTAT-1" DEVICE FOR STUDYING THE REACTION OF ORGANISMS TO PHYSICAL CONDITIONS ON THE PLANET MARS

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I. V. Koshelev, L. K. Lozina-Lozinskiy and M. A. Rybin

ABSTRACT: This article provides a description of the "photostat--1" device which enables us to reproduce the basic parameters of the physical environment of the planet Mars.

Any given temperature may be maintained in its working chamber and set to -35° to +30° with a maintenance accuracy of ±0.5°; it can produce a change in temperature with varying rates of cooling or heating; it can maintain any given pressure (in the low-pressure chamber) in a range from 5 to 95 mm Hg at an accuracy of ±2 mm Hg. In this way a stream of air or any other nonaggressive gas mixture is ensured. The system of ultraviolet filters in the device permits irradiation with the following individual lines of the ultravoilet spectrum: 1-253.7; 280 and 356 nm, 220-320 nm by the aid of 2 to 3,500 $\mu W/cm^2$; it is possible to maintain illumination in limits from 5,000 to 60,000 lux with a variation factor of ±10%. All operational conditions of the device are maintained automatically for given programs and are recorded by automatic recording devices.

One of the problems of exobiology is the determination of the possibility /296
of life on other planets of the Solar System on the basis of ability or organisms to adapt to varying conditions of external environment. Therefore the experimental-ecological method of investigations which permits clarification of the reactions of living systems to external influences and, in particular, to the physical conditions of the planet Mars is in wide use at the present time.

Among extremal factors for terrestrial organisms are in particular ultrahigh and ultralow temperatures, low atmospheric pressure (lower than 100 mm Hg), low maximum amounts of oxygen for aerobes, a high percentage of carbon dioxide in the atmosphere, a high density of ionizing radiation and great intensity of shortwave ultraviolet (UV) radiation, and the totality of these factors.

In order to study the reaction of organisms to these influences we used different chambers and devices which simulate extraterrestrial conditions to a

greater or lesser degree, chiefly those which are known for the planet Mars (Zaar, 1966; Zhukova et al., 1965; Davis et al., 1959; Fulton, 1960; Hagen et al., 1964; Kooistra et al., 1958; Hawrylewicz et al., 1966, 1967; Roberts, 1962; Young et al., 1968).

Existing devices for the simulation of the physical Martian environment by no means completely satisfy modern requirements. The use of only closed systems in which organisms are tested in an unchanging gas medium is insufficient for ascertaining the possibility of the prolonged existence of organisms in a given medium because there is a buildup of toxic products of metabolism and the absorption of oxygen from the environment as a result of the vital activities of organisms in such chambers.

In addition, on the basis of numerous exobiological investigations in which Martian environmental conditions were simulated, it can be seen that in the chambers created for this purpose either the gradual daily course of temperatures was not reproduced in the given program, or changes in illumination and intensity in the visible and UV-ranges were not considered; also the role of the continuous solar spectrum in the vital activity of organisms was not investigated. In the majority of devices the entire set of Martian conditions cannot be reproduced. Therefore, an important task in the area of exobiology is the simulation, insofar as possible, of the totality of Martian environmental conditions.

Below we present a description of the "Photostat-1" device, in which the physical conditions of the planet Mars are relatively completely simulated and by the aid of which we can study the action of different factors of the environment on cells and organisms.

The "Photostat-1" consists of a working chamber with a volume of 200 ℓ (Figure 1), a conditioner (Figure 2), a low pressure chamber (LPC) with a volume of 18 ℓ (Figure 1), a low-pressure system (Figure 3), a system of sources of UV-radiation and visible light (Figure 2), an infrared filter with a heat exchanger and a control panel (Figure 4). /298

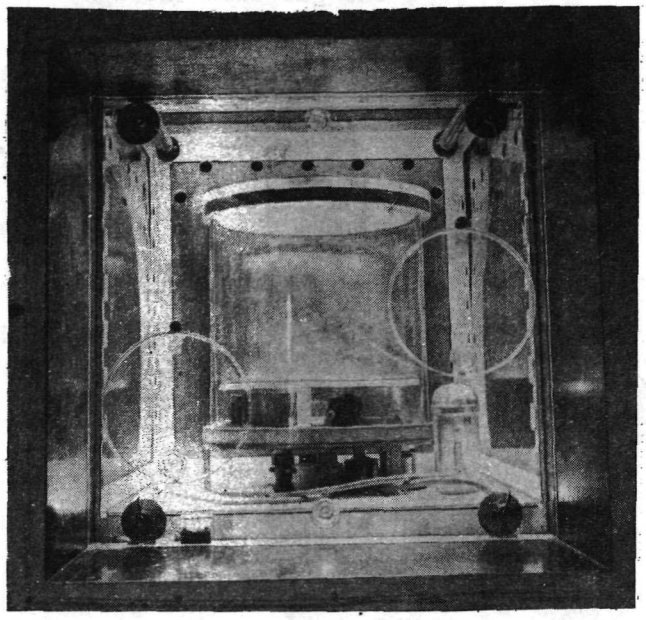

Figure 1. Working Chamber of the "Photostat-1" With a Low Pressure Chamber (LPC) (In the center).

One of the most important units of the "Photostat-1" unit is the low-pressure system. It consists of a low-pressure chamber (LPC), a vacuum pump, and a system for automatic control. The basic task of this unit is maintenance of an assigned vacuum and creation of a stream of gas through the low-pressure chamber during the experiment. The system ensures performance of either of the two operational procedures: 1) entrance of atmospheric air into the LPC, 2) entrance of gases or their mixtures from the cylinders.

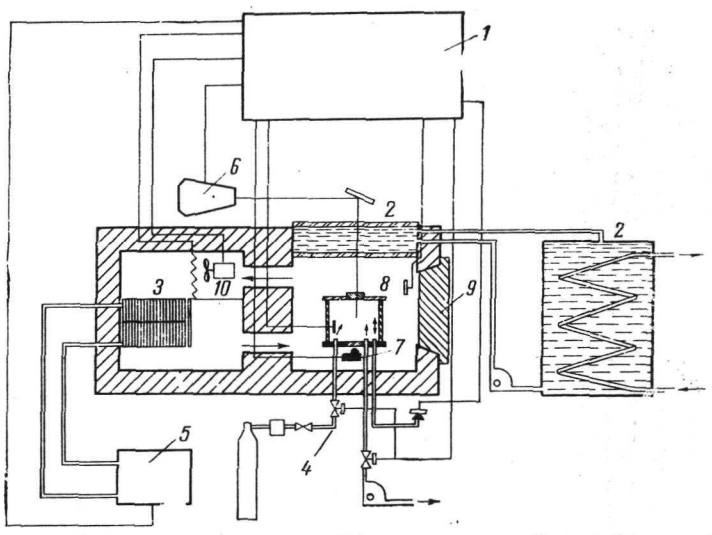

Figure 2. Layout for the Positioning of the Basic Units of the "Photostat-1" Device (Cross-Section). 1, Control panel; 2, Infrared filter with heat exchanger; 3, Conditioner; 4, Low-pressure system; 5, Cooling unit with evaporators; 6, Source of visible and ultraviolet light with a mirror; 7, Sensors for ultraviolet radiation; 8, Temperature sensor unit; 9, Door to the working chamber; 10, Heater with blowers; Arrow, Path of air from the conditioner to the working chamber.

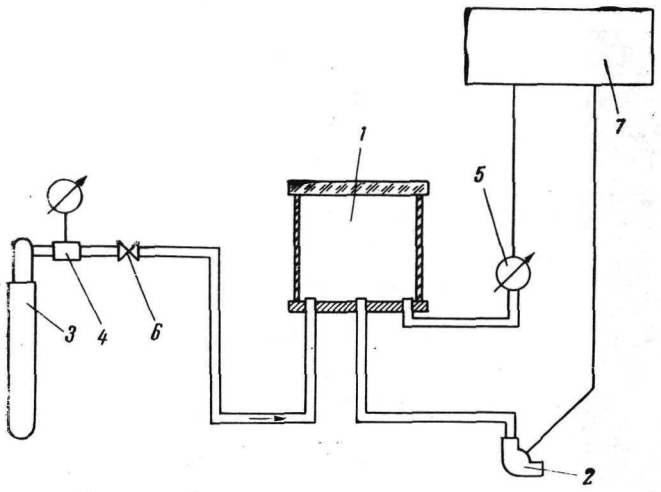

Figure 3. Layout of the Low-Pressure System. 1, Low-pressure chamber; 2, Vacuum pump; 3, Cylinder with gas; 4, Reduction valve with manometer; 5, Regulating manometer; 6, Microstopcock; 7, Control panel.

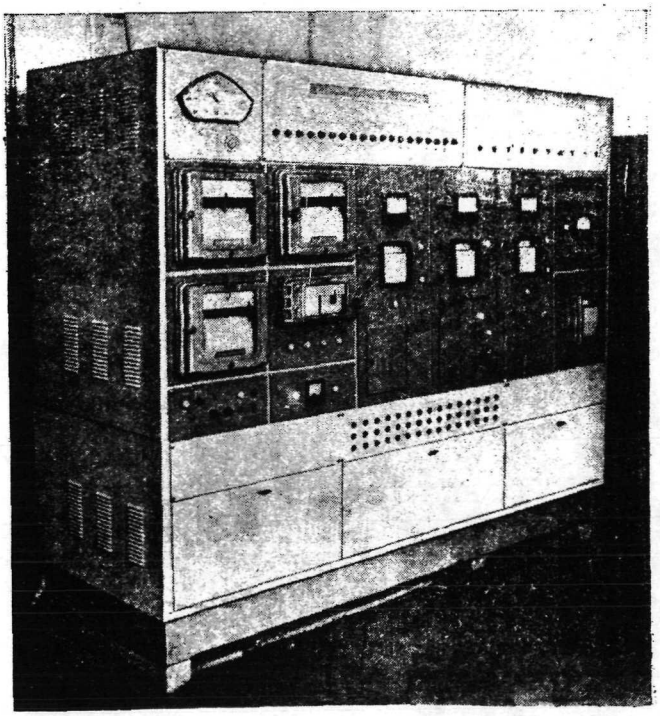

Figure 4. Control Panel of the "Photostat-1."

The gas flow through the LPC is achieved by aid of valves and microstopcocks, a vacuum pump, and a system for automatic regulation of the flow of the gas mixture.

The prolonged use of a low-pressure system under different operating conditions showed that it enables them to conduct a continuous pulsating reconditioning of gas into the LPC with a change in the flow rate of the gas mixture through the system, and it also ensures maintenance of any assigned pressure in the LPC in a range from two to 95 mm Hg with an accuracy of ±2 mm Hg.

The automatic regulation system in the LPC allows one to make from one to ten emptying and filling cycles each minute (see Figure 1 on p. 114).

As a result of these experiments it was determined that unicellular organisms can live and reproduce under conditions of an open system with identical partial pressure

of oxygen while maintaining the oxygen at a factor of 10^3 less than during the maintenance of this gas in a closed system with limited volume (2.5 ℓ) (Bychenkova et al., 1968), because of the fact that the organism and the medium represent an open system. The LPC system enables one to constantly renew the gas composition, not allowing metabolic products to build up in the chamber, and is an open system with all the advantages of the latter.

The temperature unit of the "Photostat-1" device consists of a heating and cooling system. Air of the necessary temperature is supplied along a supply line from the conditioner into the working chamber, where temperature sensor units are located. One temperature sensor unit is placed directly in the working chamber, and the other is placed in the LPC (Figure 2), which enables one to follow the change in temperature throughout the test. A change in temperature is recorded on automatic recording devices, which are installed in the control panel.

The temperature program is assigned no less than one day ahead of time by the aid of an RU5-01 instrument. The single-stage refrigeration unit ensures cooling of the air in the working chamber down to -35°.

The automatic regulation system provides maintenance of any assigned temperature in the working chamber within a range of from -35 to +30° with an accuracy of ±0.5°, and it also ensures change in temperature with different cooling-heating rates: cooling at a range from +30 to 0° with a maximum rate of 22° an hour; in a range from 0 to -25° with a maximum rate of 16° per hour; and -25 to -35° with a maximum rate of 5° per hour; heating in a range from -35 to +30° with a maximum rate of 90° an hour.

DKSSh type xenon lamps have been widely used in recent years because of the fact that the composition of their spectrum is close to the solar spectrum beyond the limits of the Earth's atmosphere, from 200 to 800 nm.

The DKSSh type xenon lamps radiate a great amount of infrared radiation (IK) which causes a significant increase in temperature in sealed chambers (Borisov et al., 1966). To absorbe the infrared radiation we used a water filter which used distilled water that had a double role; on the one hand it absorbed the infrared radiation, and on the other hand it allowed shortwave

ultraviolet rays (220-300 nm) to penetrate into the working chamber of the "Photostat-1."

Three xenon lamps (DKSSh) were located on top of the device. One DKSSh lamp was located in the center of the infrared filter. The rays from this lamp which were reflected from an aluminum mirror entered the working chamber through quartz windows in the glass bottom of the infrared filter. The shortwave ultraviolet rays (200-300 nm) entered the working chamber through quartz windows in the cover of the LPC and reached the bottom. The two other lamps served as a source of visible rays and their rays penetrated the working chamber through the glass of the infrared filter which cuts off the greater part of the ultraviolet rays.

The system for automatic regulation of illumination made it possible to carry out a gradual change in illumination from 5,000 to 65,000 lux with a nonuniformity of ±10-15% (at the bottom of the LPC). The minimum temperature in the working chamber at which ultraviolet irradiation and illumination of maximum intensity is possible is -20°.

The system of interchangeable light filters enables one to produce irradiation with different lines of the ultraviolet spectrum (253.7; 280 and 365 nm; 220-380 nm with a dose rate from two to 3,500 $\mu W/cm^2$.

Control of the ultraviolet radiation dose is achieved by the ultraviolet meter and the ultraviolet dosimeter which automatically turns off the ultraviolet radiation source after the maximum assigned dose is reached.

A distinctive feature of the "Photostat-1" device is that it enables one to influence different organisms both by different physical factors and by a set of physical factors according to a program arranged ahead of time and according to the desire of the experimenter, and it enables one to change the parameters within wide limits. In case one of the units of the device malfunctions the power supply to the damaged unit is automatically shut off and a light comes on which indicates the trouble point and its cause.

Thus, the "Photostat-1" device enables one to simulate comparatively accurately pressure, illumination by visible light (up to 60,000 lux), to

expose biological objects to ultraviolet rays(220-400 nm) and to reproduce a daily temperature cycle which is characteristic for the planet Mars.

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RESISTANCE OF UNICELLULAR ORGANISMS TO ULTRAVIOLET RADIATION IN RELATION TO THE PROBLEM OF THE EXISTENCE OF EXTRATERRESTRIAL LIFE

L. K. Lozina-Lozinskiy

ABSTRACT: The ultraviolet radiation of the Sun is one of the most important factors which determine the existence of life on the Earth, beyond the Earth, in space and on planets which do not have a gas environment which absorbs shortwave ultraviolet radiation.

Rays in the range λ = 240-280 nm, which are selectively absorbed by proteins and nucleic acids, have a vigorous harmful action on terrestrial organisms and therefore the opinion is widespread that life is limited by shortwave ultraviolet radiation. As studies in the author's laboratory and by a number of other authors show, terrestrial organisms and cells have properties and abilities which enable them to have a resistance to shortwave ultraviolet radiation and increase their resistance to it. Examples are presented of the capability of unicellular organisms for "dark" reparation, the important role of pigment and protective substances which are formed in an organism and substances present in the environment which act as shields and it also indicates the possible importance of adaptation of cells to ultraviolet rays. It was concluded that various methods of protection from ultraviolet radiation and adaptation may arise in the process of evolution as a result of which life is not limited by the action of shortwave radiation of the Sun.

Explanation of the possibility of adaptation of biological objects to such /302
a factor as the shortwave region of the ultraviolet radiation of the Sun is of great interest for exobiology. Terrestrial organisms, being protected from ultraviolet rays shorter than 290 nm, have not adapted to it. As numerous experiments have shown high doses of shortwave ultraviolet radiation (λ = 240-280 nm) killed all bacteria both in the active stage and as spores, and also other microorganisms and cells of animals and plants.

The highly injurious activity of ultraviolet rays (lethal, mutagenic, antimitotic action) are related to their selective absorption by the most important molecules which comprise living matter. The maximum absorption of nucleic /303

acids is in the range of 260-265 nm; proteins, because they contain a peptide bond, absorb rays with $\lambda < 200$ nm, and due to the presence in their composition of aromatic amino acids they also absorb rays in the range from 270-290 nm. The absorption of carbohydrates and lipids is in the range of the vacuum ultraviolet ($\lambda < 185$ nm). Different enzymes, vitamins and pigments absorb primarily the longwave ultraviolet radiation and also the visible light.

Therefore, although only about 6% of the total energy of the optical radiation of the Sun is shortwave radiation (290-10nm), it is one of the principal factors which prevent life. In relation to the possibility of organisms adapting to differing extremal actions, including radiation factors, and considering the history of Earth, such an assumption seems quite categorical to us especially when we think of the number of theoretical considerations and experimental data of recent years. Life on Earth was generated at the same period in its history when oxygen and ozone were lacking in the primary atmosphere (regenerative period) and when the range of ultraviolet rays most dangerous to biological objects reached the surface of our planet.

In the opinion of many researchers (Oparin, 1957; Pavlovskaya, 1959; 1968; Pannamperuma, 1966; Pasynskiy, 1964; Terenin, 1959; Khenokh et al., 1968), shortwave ultraviolet radiation may play a great role in the synthesis, decomposition and evolution of organic compounds which are part of the structure of living organisms, including amino acids, and also such protective substances as pigment (Gaffron, 1966). In the opinion of M. N. Kamshilov (1965, 1968), the protoorganisms were formed during the action of ultraviolet radiation of the Sun and unique rudiments are retained in them which reflect this stage in the evolution of organisms. It is possible that the origin of unicellular organisms in an aqueous medium even occurred during the regenerative period of the existence of the Earth. Because the formation of oxygen and ozone in the atmosphere of the Earth evidently lasted a long time, this stage in the existence of the Earth was possibly the most favorable for different adaptation of primitive organisms to the weakened action of ultraviolet radiation of the Sun.

As investigation of the effects caused by ultraviolet radiation in modern terrestrial unicellular organisms confirms, the organisms have significant possibilities which permit them to withstand the destructive action of this /304
factor. As the oxidative period originated under conditions of an atmosphere which retained the most harmful range of the ultraviolet radiation, the necessity of such an adaptative mechanism disappeared, and did not become an object of natural selection and progressive evolution. As a result the organisms could lose their former resistance. Thus the subject of the present article is the result of certain projects of our laboratory in a study of the causes which intensified the ability of cells to withstand ultraviolet rays.

As an analysis of the reaction of many biological objects to ultraviolet rays shows, the sensitivity of this factor fluctuates within extremely wide limits. If we accept the dose which causes death in 90% of the cells (LD_{90}) as a criterion for sensitivity, it will be least for cells of mammals and certain bacteria (4-250 ergs/mm^2), and greatest for Protozoa (infusoria and flagellates, 5,000-12,000 ergs/mm^2). For the overwhelming majority of bacteria LD_{90} does not exceed 500 ergs/mm^2; various fungi are significantly more resistant: some of them withstand doses of 800-3,000 ergs/mm^2. Within one species of these organisms of *Rodotorula* the sensitivity of ultraviolet irradiation varies by thirty times (Solntseva, 1967).

The colorless marine flagellate *Bodo marina*, for which LD_{90} reaches 112,000 ergs/mm^2 (Kamshilov, 1963, 1965) is the most resistant to ultraviolet rays of the objects studied. According to Kamshilov marine microorganisms and Protozoa generally are distinguished by a high level of resistance to ultraviolet radiation. However, this is hardly true for unicellular algae (McLeod and McLachlan, 1959).

In certain pigment-containing objects ultraviolet irradiation does not cause such severe damage as in objects which do not have pigment. Evidently certain pigments act as a screen which is not transparent to ultraviolet rays. Differences in resistance between colored and colorless breeds of microorganisms may reach a factor of 10^2 (Imshenetskiy, 1964). Examples of pigments which

have protective properties are flavones and anthocyans. However, vegetative cells which are rich in chlorophyll and carotinoids do not have any especially high resistance to ultraviolet rays (Dubrov, 1963). For example, the LD_{90} for *Chlorella vulgaris* does not exceed 2,500 ergs/mm^2 (Kvitko, 1963). Cells of *Euglena gracilis*, which contain chlorophyll, and decolorized forms isolated from the same culture almost do not differ in their sensitivity to shortwave ultraviolet radiation (Klein et al., 1963 and our unpublished data). K. A. Samoylova (1967) shows that the pigment melanin causes a vigorous protective effect. For example, the mold fungus *Aspergillus niger* among this group has an especially high resistance, surviving under doses of 16,000 ergs/mm^2, while the related species of *A. nidulans*, which has no melanin, has a resistance to /305
ultraviolet rays which is two times less. According to the data of Gize (1953), the infusoria *Fabrea salina*, which has a dark pigment, is almost nine times more resistant to ultraviolet rays than nonpigmented infusoria. The protective role of pigmentation as manifested in varying degrees in different clones of the infusorian *Blepharisma undulans* is particularly convincing (Giese et al., 1965).

In connection with the screening action of certain pigments we must emphasize the physical or biophysical protection of the cells during their processing by certain unstained substances or when the latter are lacking in the medium. It was shown that ultraviolet irradiation in solutions of substances which are very important in the life of cells, such as cysteine, tryptophan, glutothione, tyrosine, phenylalanine, thymine, cytosine, uracyl, ATP, etc., sharply reduce the harmful action of ultraviolet rays. This is related to the fact that, by absorbing ultraviolet rays, these substances will serve as a physical screen above the sensitive components of cells (Samoylova, 1963; Whitehead, 1952). This was shown experimentally in *Paramecium caudatum* (Samoylova, 1963). However, in the case of ATP this protective action was manifested not only by its presence in the medium with infusoria but also when the latter were previously retained in the solution with ATP, and then were irradiated without it in the pure medium. In the opinion of K. A. Samoylova (1963) in this case it is most probable that ATP served as a physical screen as a result

of penetration into the cell or absorption on the surface of the infusoria rather than that these substances (and possibly others) entered into the photochemical reactions within the cell.

Certain microorganisms and Protozoa pass through the stage of spores or cysts during their life cycle. In this state they reveal a higher level of resistance to ultraviolet rays and to other influences. The resistance of spores on the average is two times greater than that of actively metabolizing cells. In Protozoa this discrimination towards ultraviolet rays is expressed even more vigorously: dormant cysts of the soil infusorian *Colpoda maupasi* are 3.5 times (Lozina-Lozinskiy et al., 1968) and amoeba cysts are 33 times (Dreyer, 1903) more resistant than the active forms. The resistance of spores and cysts to various types of harmful influences is usually explained by the low amount of water in them and by the reduction of metabolic intensity. However, for such a factor as ultraviolet rays, whose effectiveness is directly related to the degree of penetration of an object, the presence of special membranes or capsules in their cysts and spores must be no less essential.

The diffuse nature of the distribution of DNA in cysts, which serves as an intercellular target for the shortwave ultraviolet rays, is evidently essential. An increase in resistance to ultraviolet rays occurs at the same time as cessation of the synthesis of RNA in the process of the formation of /30
spores (Romig et al., 1957). Undoubtedly the peculiarities of the nuclear apparatus of cells have a direct relationship to their resistance to ultraviolet radiation. It is not by chance that the greatest resistance to this factor among unicellular organisms characterizes the class of infusoria which have a high degree of ploidy in the cell nuclei. During transition from the haploid state to the diploid state (in certain strains, to the triploid state) the resistance to ultraviolet rays increases by 2-2.5 times as is shown in tests with homozygotic strains of yeast which differ in number of chromosome sets (Korogodin et al., 1963; Laskowski, 1960; Sarachek et al., 1953). The further increase in ploidy of the yeast cells is not accompanied by an increase of resistance. Cells of certain clones of infusoria which contain twice as much DNA or substrains of cell populations of mammals also

demonstrate a significantly higher level of resistance to ultraviolet radiation (Samoylova et al., 1968). Reduction of sensitivity of cells to ultraviolet rays as the amount of DNA in them increases can be observed when cells are irradiated at different stages of their mitotic cycle. Almost all unicellular objects reveal a greater resistance to ultraviolet rays in the final phase of DNA synthesis or immediately after its completion. At this period cells contain twice as much DNA and manifest a twofold higher resistance to radiation (Samoylova, 1967).

It is interesting that simultaneously with the increase in resistance to the lethal action of ultraviolet radiation resistance to the mutagenic action is also acquired. It increases by almost ten times in infusoria (Kimball, 1963). As exemplified by experimentally established facts the basic cause of an increase in the resistance of polyploids is explained by the presence of a more vigorous system of reparation of radiation damage in them (Korogodin et al., 1962, 1962; Oparin, 1957). Not only the amount of DNA but also the structural peculiarities of its macromolecules have an effect on the resistance of cells to ultraviolet radiation. Thus, it was established that resistance of unicellular organisms to ultraviolet radiation increases as the thyminic nucleotides in their DNA decreases, which is also true of the adenine nucleotides which are complementary to them and are characterized by high photochemical activity (Samoylova, 1967).

Replacement of the thymine by halogenized analogs which are distinguished by a greater photochemical activity is accompanied by a reduction in the resistance to ultraviolet radiation in the 240-280 nm range by 7-8 times, and to radiation with λ = 240 and 280 nm by 12-700 times.

The level of resistance of unicellular organisms to ultraviolet radiation depends to a great degree on their physiological state, which is determined by a combination of many factors, the phase of the mitotic cycle, culture growth, nature of the oxidative metabolism, and also by culturing conditions before and after irradiation.

The relationship between sensitivity to ultraviolet rays and the gas composition of an atmosphere is of interest to exobiology but has been studied

very little, although it is known that an oxygen effect during ultraviolet radiation is present. However, this does not mean that the resistance of unicellular organisms to ultraviolet rays remains constant under varying conditions when the intensity of metabolism and the oxygen requirement are not identical. In the infusoria *P. caudatum* a resistance to ultraviolet rays is intensified on the second-fourth days of the cycle of development of a culture, that is in the first half of the phase of the exponential growth, or in other words during the period of the most intense respiration (Lozina-Lozinksiy et al., 1963; Samoylova 1966). Thus, adaptation to ultraviolet rays could go not only in the direction of intensification of metabolism and reduction of sensitivity to metabolic inhibitors, for example, to α-dinitrophenol, but also to the advantage of anoxybiosis, a transition toward glycolysis. *E. coli* bacteria during cultivation under anerobic conditions, become more resistant to ultraviolet rays (Hodgins et al., 1963).

The kinetic effect of ulttaviolet irradiation, as follows from a test on quite different objects, manifests itself to a greater degree in cells which are maintained under anoxic conditions or have been treated by respiratory toxins. One recalls the fact that the amount of chromosomal aberrations in pollen cells of spiderwort is identical during irradiation in oxygen and in nitrogen (Kirby-Smith et al., 1957); however, in the latter case a higher rate of unrecombined breaks is noticed. This can be seen as an index of the fact that reparation of damage to genetic material is suppressed under anaerobic conditions.

The problem of the resistance of cells to ultraviolet radiation is very complex and has been studied very little. In this regard it is necessary to emphasize that the resistance to ultraviolet rays is affected by the level of the physiological state of the cells in the organism, whose general resistance may be reinforced by the action of nonspecific agents. Thus, substances which do not absorb ultraviolet rays may increase the resistance of cells to damaging influences, including radiation. It was shown in cells of the ciliate epithelium and in infusoria that the resistance to ultraviolet rays is intensified by calcium ions (Levin, 1960; Samoylova, 1963), by glycerin (Samoylova, 1963) and by such substances of pharmacological action as dibasole and demisole

(Levin, 1962, 1964), which are absorbed by cells. It is known that these substances increase resistance to many agents (Rozin, 1967).

One of the most important conditions for adaptation and resistance of biological objects to extremal factors is the capacity of living systems to repair damages which occur. This capacity is very clearly expressed in the majority of the cells and unicellular organisms which we studied that were irradiated by ultraviolet rays. If the irradiation dose is not too great, the cells can repair the damage which occurs, basically due to two systems of regeneration which are connected to the activity of certain enzymes. One of the systems which was studied in detail in microorganisms acts under conditions of a non-nutritive medium either in the presence of inhibitors of protein synthesis in the medium, or at high temperature. The non-nutritive medium is necessary for delaying the first post-radiation division, which gives the cells time for regeneration. /308

This process is related to the fact that elimination of thymine dimers from the DNA molecule occurs in the cell. These dimers are formed during the action of shortwave ultraviolet radiation on the nucleus. This process is known as dark regeneration (DR), because it proceeds without the participation of light. DR increases the survival rate of cells and promotes an increase of resistance to ultraviolet radiation by tens and hundreds of times.

In the infusorian *P. caudatum* the DR process evidently occurs somewhat differently than in microorganisms because reactivation is more active and the mortality is lower if the paramecia are placed for one to two days after irradiation into a medium with yeast which serves as food for them. First of all the phagocytic function, which is very sensitive to ultraviolet rays, is regenerated in the infusoria (Lozina-Lozinskiy, 1960). Evidently the regeneration system is called upon to protect the cells not so much from ultraviolet rays, whose intensity and special composition on our planet is not dangerous for biological objects, as for maintenance of the known constancy of genetic material of cells under conditions of the continuous action of different environmental factors on them. The adaptive importance of this system of nonspecific regeneration is obvious.

Another regenerating system may operate against the background of DR — photoreactivation (PR), which is characterized by the fact that as a result of illumination of ultraviolet-irradiated cells by visible light or by rays in the longwave range of the ultraviolet spectrum cell damage is reduced and is expressed in a cessation of division and a reduction in remote mortality. The effect of PR in Protozoa significantly exceeds the effect of dark reactivation and the infusoria may divide in the presence of light at sublethal doses. Photoreactivation is characteristic for the majority of organisms, and its absence is seen as the result of a mutation of the corresponding gene.

PR was achieved by the aid of a group of enzymes some of which resemble cytochromes. Violet and blue are the most active photoreactivating rays, but /30 it has been shown that the longwave range of invisible light, beginning with green rays, is also capable of causing the PR effect (Lozina-Lozinskiy, 1960, Lozina-Lozinskiy, Zaar, 1963; Klein, 1963). Although in this case the PR action is significantly weaker and takes place only in cells which have been subjected to irradiation by nonlethal UV doses, its role at high intensity of the longwave range of the visible sunlight in PR is determined by the ratio of energy in this range and the shortwave range of the ultraviolet radiation which is still insufficiently understood. Under exprimental conditions the optimum ratio is 10-100:1, which is close to the natural ratio.

Outside the Earth's atmosphere where living objects must be irradiated simultaneously by ultraviolet rays as well as by the remaining range of solar radiation, it is important that the PR proceed simultaneously with the action of ultraviolet rays. As tests by E. I. Zaar have convincingly shown, during simultaneous action of visible light and ultraviolet rays (dose of 120 $\mu W/cm^2$) on infusoria where damaging effects might not be present, and under conditions of continuous irradiation by ultraviolet and visible light, infusoria preserve their normal rate of division. M. M. Kamshilov (1965) indicated a similar possibility, after having studied the action of ultraviolet irradiation and visible light on microbiocenoses of marine Protozoa for a period of many years.

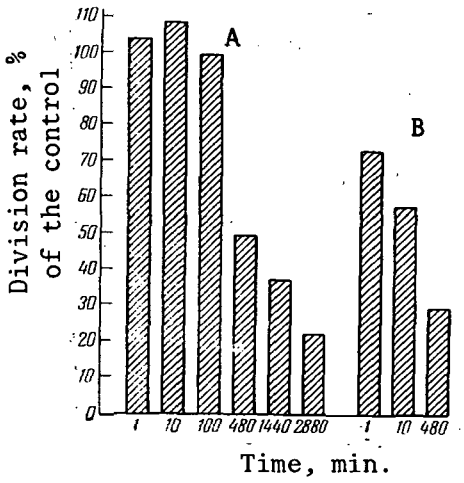
Rays longer than 300 nm have a reactivating effect if the cells irradiated by them are subjected to the action of rays with λ = 254 nm, both before irradiation and after irradiation (Jageer, 1964). Antagonistic relations and the interaction in various ranges of the solar spectrum are not limited by this. In the case of complex irradiation of the bacteria *E. coli* of group B by α-particles and by ultraviolet rays, sensitivity to ultraviolet radiation (λ = 254 nm) increases. Evidently the indicated change in radiosensitivity is related to suppression of the DR capacity of bacteria (Zavol'naya, 1966) with radiation damage. Ye. S. Zavol'naya established that the capacity for PR was also suppressed as a result of previous α-irradiation (Zavol'naya, 1968).

In order to understand the role of ultraviolet radiation as a space factor which acts on living objects it must be determined whether the existence of biological systems is possible during prolonged irradiation, because it is unlikely that short-term influences of ultraviolet rays play a role in space. This is particularly important because continuous single or fractionated irradiation causes a most serious injurious effect due to the cumulative action of ultraviolet rays, which it is impossible for an organism to overcome (Lozina-Lozinskiy, 1960). Therefore the existence of cells during prolonged irradiation is determined not only by the time factor but also by the amount of dose received and the capacity to reactivate during irradiation and after it. /310

It has been shown in the case of paramecia (*P. caudatum*) that repeated irradiation, especially in a series at short time intervals (hours, days), intensifies the damaging effect (Lozina-Lozinskiy, 1960). Infusoria, which have endured maximum doses and reproduced normally, are characterized by an increased sensitivity to ultraviolet rays for a month and longer. With small doses the time of increased sensitivity is abbreviated to 2-3 days, and resistance to ultraviolet rays may become higher than before the first irradiation (Lozina-Lozinskiy, 1960, 1968).

The role of single and fractional irradiation of paramecia as well as the time factors were studied in more detail by E. Zaar (1964, 1965, 1966). It is shown in the drawing that, beginning with a radiation duration of 480 minutes and longer, with an identical dose of 120 $\mu W/cm^2$ the resistance of paramecium,

judging by the reduction in division rate and increase of mortality, drops sharply. It is also observed with a larger dose of 960 $\mu W/cm^2$. Thus, if ultraviolet rays (λ = 254 nm) penetrate into a cell whose mitotic cycle duration fluctuates from 18 to 24 hours, then one-time irradiation with a high dose is significantly less dangerous than irradiation of low intensity which continues for eight hours or more. There are not yet sufficient data to explain these defenses and the degree of damage. We can suppose that in the case of prolonged irradiation with a lengthy cellular cycle, the action of ultraviolet rays affects all stages including the most sensitive stages of the cellular cycle, but during short-term irradiation part of the infusoria escape this effect. There is another possible explanation, according to which, due to the continuous prolonged action of ultraviolet rays, the cells lose their capacity to repair damage, which results in a cumulative effect.



Division Rate of Infusoria (*P. caudatum*) for the First Six Days Following Irradiation by Ultraviolet Rays. A, Dose of 120 $\mu W/cm^2$ (718 $ergs/mm^2$); B, Dose of 960 $\mu W/cm^2$ (5,744 $ergs/mm^2$).

Let us imagine what radiation conditions are like on Mars. There, organisms which are on the surface must experience radiation for a period of many hours, but one which is comparatively weakened by the great distance from the Sun, by absorption of the atmosphere which consists primarily of CO_2, and possibly by other factors. Consequently, the intensity of ultraviolet radiation on Mars must be significantly less than on our planet during its regenerative period, when the energy of ultraviolet radiation of the Sun is in the range of $\lambda < 250$ nm or 660 kcal/cm a year (Pavlovskiya et al., 1968).

In this regard the role of photoreactivation may have an especially great importance under conditions on Mars. In addition to the indicated factors which

affect the resistance of biological objects to ultraviolet rays, other forces also act (for example, temperature) but their role is still quite unclear.

The study of the causes of resistance, damage and processes of regeneration, chiefly in microorganisms and Protozoa during irradiation by shortwave ultraviolet radiation indicates that living systems have numerous possibilities for increasing their resistance and adaptation to ultraviolet rays. This is evidenced by the huge range of sensitivity to radiation in various organisms (whose causes have still not been examined), the capacity for adaptation, formation and buildup of substances which absorb ultraviolet rays and which act as a shield for the sensitive targets in the cell, and the great possibilities for reparation of the ultraviolet damage, a factor which is especially essential.

The capacity of cells for photoreactivation must play a very important protective role beyond the limits of the Earth's atmosphere in space. We can suppose that early terrestrial organisms had a more vigorous PR system which was necessary during the regenerative period and in the beginning of the oxidative period of the Earth's history.

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THE ACTION OF ULTRAVIOLET RADIATION ON AMINO ACIDS AND PEPTIDES IN VARYING GAS ATMOSPHERES IN THE PRESENCE OF SALTS AND OXIDES OF METALS

M. A. Khenokh, N. P. Bogdanova and Ye. M. Lapinskaya

ABSTRACT: The article shows that various reactions occur due to the ultraviolet effect in solutions of amino acids and peptides which have been sensitized by different cations, oxides of metals and polyphosphoric acid as, for example, breaking of the carbohydrate chain, hydroxylation of the aromatic ring, deamination, the formation of organic acids and carbonyl substances. New amino acids are also formed. The smaller the carbohydrate chain the less will be the quantum decomposition yield ($\Phi(-m)$) of amino acids and peptides (with an identical acyl radical). It makes the supposition that the initially sensitized peptides in the prebiological era of the existence of our planet consisted of amino acids with a shorter chain of carbohydrate atoms.

The data shows that such ores as hematite (Fe_2O_3), rutile (TiO_2), zincite (ZnO), silica (SiO_2) and various cations might have a catalytic effect on photochemical conversion of abiogenically synthesized amino acids. Moreover, the low temperature (-78°) did not prevent photolysis from occurring.

In connection with the appearance and development of the new division of /313
exobiology within biology, the number of publications related to problems of the origin of life on Earth and possibilities of its existence on other planets suddenly increased. In many experiments carried out at the molecular level the physical conditions which were the dominant ones on the Earth in the prebiological period of its existence were reproduced. Under such conditions authors attempted to synthesize the biochemically important compounds necessary in the future for construction of cellular material out of the simplest substances. Important advances have been made in this area (Pasynskiy and Pavlovskaya, 1964; Rosnay, 1967). In particular the abiogenic synthesis of aliphatic and aromatic amino acids was accomplished. The importance of ultraviolet radiation under whose effect reactions of this kind took place is

especially emphasized. At present we have enough material to conclude positively that similar syntheses could take place on other planets. This is confirmed by the results of chemical analyses of different meteorites (Vdovykin, 1967; Hayes, 1967). However, one should note that amino acids could decompose due to the effect of radiation (one of the factors which cause their formation) to produce a number of new substances. Amino acids which were synthesized in the original atmosphere of our planet then entered the hydrosphere, the saline medium — the seas and oceans.

In addition to the penetrating ultraviolet radiation, different soluble salts, polyphosphates, and oxides of metals would emerge as protosensitizers /314 which catalyze or inhibit photochemical conversions of amino acids (Lapinskaya, 1967, 1969; Khenokh, Bogdanova, 1968a, b, c). In our opinion the systematic study and explanation of the causes for the resistance of different amino acids to the simultaneous action of space factors such as ultraviolet and ionizing radiation, in combination with changing temperature conditions and gas composition of the atmosphere in the presence of different photosensitizers, helps us to better understand the amino acid composition of the simplest peptides which were abiogenically synthesized in the primary geological era of the existence of the Earth. Such material is also of undoubted interest for its possible application in predicting the amine-containing substances on other planets.

Aliphatic amino acids undergo essential chemical conversions under the effect of ultraviolet (UV) radiation. In addition, there is the decomposition of the NH_2-group with the liberation of ammonia, breaking of the carbon-carbohydrate bond with the formation of carbonyl substances, organic acids and aldehydes. It was determined that ongoing photochemical reactions may be sensitized or suppressed by various cations and oxides in metals (Khenokh, ogdanova, 1968a, b, c).

Our results also showed that the longer the carbohydrate chain of the amino acid the less would be the deamination and formation of glyoxalic acid.

It is interesting that such a characteristic was detected during irradiation of amino acids by ionizing radiation. A possible explanation for these facts, which is based on the self-protection of decomposition products, is given in

our earlier published papers (Khenokh, Lapinskaya, 1955, 1958). The products which arise as a result of photolysis may be distributed according to decreasing concentration in the order:

$$NH_3 > CH_3 - CHO > CHO - COOH > H_2CO.$$

The experimental material enabled us to conclude that during the irradiation of solutions of NH_2 acids which contain salts, the magnitude of deamination is determined by the cation present (photosensitizer) and is independent of the length of the carbohydrate chain, while the formation of other products, for example, glyoxalic acid, depends on the length of the amino acid chain (Khenokh, Bogdanova, 1968a, c).

Cations can be arranged in the following order according to their activity and increase in the quantum decomposition yield ($\Phi_{(-m)}$) of amino acids in solutions of glycine, alanine and norleucine:

$$Cu^{2+} = \text{"mixture" (cations)} > Fe^{2+} > Mn^{2+} > \text{amino acid} > Zn^{2+} = Al^{3+}.$$

Because the above-described processes may also occur on other planets, for /315
example, on Mars, we decided to study the effect of different gas atmospheres on photochemical conversions of 10^{-2} M solutions of amino acids and peptides in the presence of certain ions of the metals of sulfate salts (10^{-2} M). The methods of our investigation were presented in earlier published papers (Khenokh, Bogdanova, 1968a, b, c). Results obtained showed that, for example, photolysis of alanines in an atmosphere of Ar and N_2, in comparison with an atmosphere of air, cause no noticeable change of deamination of amino acid.

Sharper distinctions were found in the formation of ammonia when we irradiated solutions which contained Cu^{2+} ions. The yield of ammonia into air and into Ar with an identical dose (8×10^9 ergs/cm^2) of irradiation, was respectively 8×10^{-3} M and 6.5×10^{-3} M. A reduction of deamination (to 3.2×10^{-3} M) occurred during irradiation of alanine in an atmosphere of CO_2 (on the linear section of a buildup curve). Cu^{2+} ions substantially increased

the liberation of ammonia into the air (from 4.5×10^{-3} M to 8×10^{-3} M) in comparison to CO_2 and increased it to a lesser degree in N_2 and Ar.

We determined that the gas which was saturating the solution also had a noticeable effect on the photochemical conversions of alanine which led to the formation of glyoxalic acid. During irradiation in air (dose of 8×10^9 ergs/$/cm^2$) we found 0.82×10^{-3} M of acid, while when using the same dose in N_2, Ar and CO_2 the amount of glyoxalic acid was respectively, 0.25, 0.55 and 0.28×10^{-3} M.

By irradiating alanine in different gases (Bogdanova, Khenokh, 1968), we were able to establish that Ar and CO_2 vigorously reduce the yield of acetaldehyde while N_2 has practically no effect (Figure 1). The formation of formaldehyde was sharply reduced in an atmosphere of N_2, Ar and CO_2. We found 0.25×10^{-3} M of formaldehyde during irradiation in air (dose of $8 \cdot 10^9$ ergs/cm^2) but in the presence of N_2, Ar and CO_2 we found, respectively, 0.08, 0.03, and 0.03×10^{-3} M. The number of total carbonyl substances which originated during photolysis of alanine in various gases conforms to the sequence: /316

$$\text{Air} > N_2 > CO_2 > \text{Ar}.$$

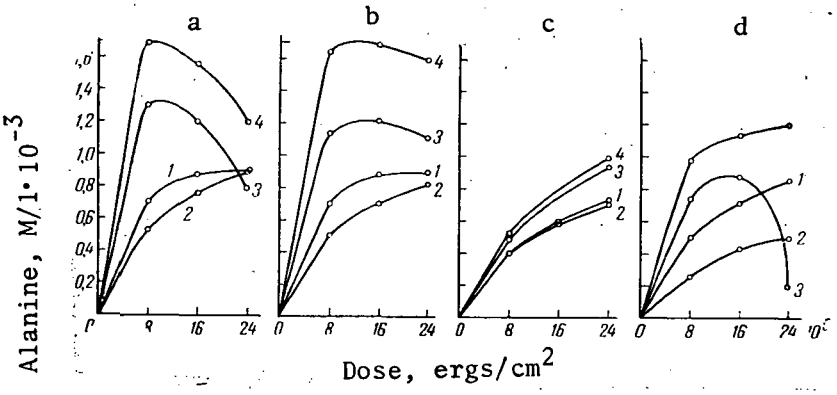


Figure 1. Formation of Acetaldehyde In the Photolysis of Alanine (0.01 M) In the Presence of Cations In Atmospheres of Various Gases. A - Air; B - N_2; C - CO_2; 1 - Alanine irradiated in the presence of cations (0.01 M); 2 - Al^{3+}; 3 - Fe^{2+}; 4 - Cu^{2+}.

The cations are arranged in the same order as in the air according to the increasing action of the $\Phi_{(-m)}$ of alanine ($\Phi_{(-m)}$ in the air = 1×10^{-3}) in the various gases:

$$Cu^{2+} > Fe^{2+} > \text{alanine} > Al^{3+}.$$

Thus, regardless of its dependence on the atmosphere in which the photolysis of amino acids occurs, the sequence in the series of cations based on their photosensitizing activity is preserved in practice. Similar results were obtained for solutions of valine and leucine. We found glycine among the products of photochemical conversion of valine and leucine. However, these solutions contained Cu^{2+} ions and a "mixture," so the photolysis of valine and leucine proceeded without formation of this amino acid. The latter fact indicates that the photooxidative reactions which occur are intensified in the presence of Cu^{2+} ions in the "mixture" and cause complete destruction of the glycine being formed.

During irradiation of the norleucine we obtained a chromatogram (Figure 2), which showed that the decomposition of the amino acid was accompanied by the /317
appearance of glycine and alanine. The addition of certain cations substantially changed the nature of the chromatograms. Thus, Al^{3+} and Fe^{2+} suppressed, respectively, the formation of glycine and alanine, but in the presence of Cu^{2+} these amino acids were generally not detected.

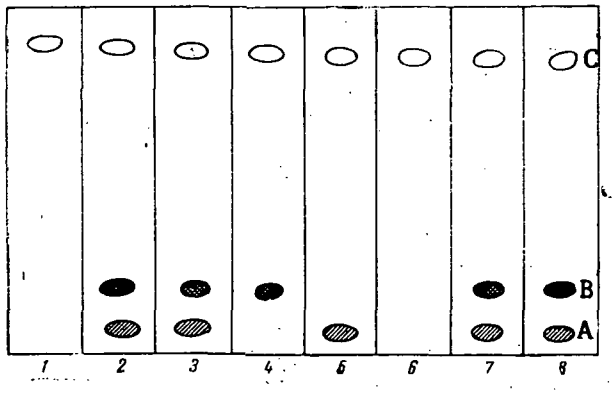


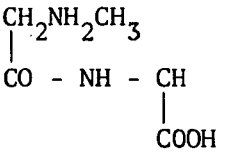
Figure 2. Chromatogram of Irradiated Norleucine (0.01 M) in the Presence of Cations (Dose was 8.0×10^9 ergs/cm^2). A, Glycine; B, Alanine; C, Norleucine; 1, Non-irradiated norleucine; 2, Norleucine, irradiated in the presence of cations; 3, Zn^{2+}; 4, Al^{3+}; 5, Fe^{2+}; 6, Cu^{2+}; 7, Mn^{2+}; 8, Mixture.

The Φ of the formation of glycine and alanine from norleucine (based on data of quantitative chromatography) was determined within the limits $0.5 - - 0.8 \times 10^{-3}$ for glycine, and for alanine it was 0.45×10^{-3}.

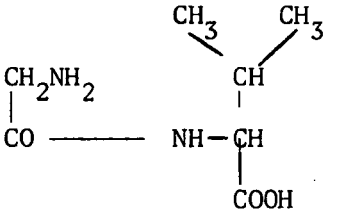
The cations can be arranged in the following order according to their effect on the Φ of the formation of glycine from valine:

Valine = $Al^{3+} > Zn^{2+} = Fe^{2+} = Mn^{2+} > Cu^{2+} >$ "mixture," i.e., Cu^{2+} and the "mixture" more energetically photosensitize the decomposition of the glycine being formed.

During irradiation of the peptides we obtained results which showed that no decomposition to free amino acids occurred due to the effect of the UV (dose $2 \times 10^9 - 6 \times 10^9$ ergs/cm^2) in the glycylalanine in the presence of cations as well as without them.

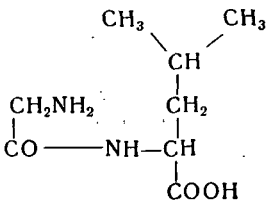


However, in solutions of glycylvaline



the same doses led to the appearance of a number of spots (Figure 3) on the chromatogram. On the chromatogram of the peptides irradiated without cations, and also of the peptide in the presence of Zn^{2+}, Al^{3+} and Mn^{2+} we detected only one additional spot, which corresponded to the reference spot for glycine. We found no free valine at this time. The valine appeared during photolysis of solutions which contained ions of Cu^{2+}, Fe^{2+} and "mixture."

The chromatogram of the irradiated glycyleucine (Figure 4) illustrates the relationship



of the photochemical conversions of peptide to the radiation dose in the presence of the cations. From the chromatogram it is evident that photolysis of the peptide proceeds with a break of the—CO–NH– bond with simultaneous formation of glycine and leucine. The presence of Zn^{2+} and Mn^{2+} in the solution suppresses cleavage of the free leucine, but Fe^{2+}, Cu^{2+} and the "mixture" catalyze the formation of alanine. With an irradiation dose of 2×10^9 ergs/cm^2, Al^{3+} ions inhibit the appearance of free amino acids. An increase of the dose (4×10^9 ergs/cm^2) enhances preservation of the leucine spots, if the photolysis proceeds in the presence of Fe^{2+}, Cu^{2+} and the "mixture." In addition, there is a decomposition of leucine in the initial solution and of alanine in the presence of the "mixture."

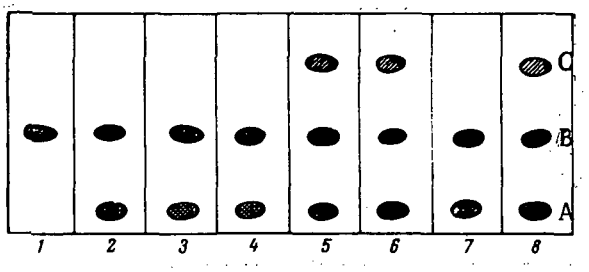


Figure 3. Chromatogram of Irradiated Glycylvaline (0.01 M) in the Presence of Cations (Dose is 2-6 × 10^9 ergs/cm^2) A, Glycine; B, Glycylvaline; C, Valine; 1, Non-irradiated peptide; 2, Peptide irradiated in the presence of cations; 3, Zn^{2+}; 4, Al^{3+}; 5, Fe^{2+}; 6, Cu^{2+}; 7, Mn^{2+}; 8, Mixture.

Increasing the dose to 6×10^9 ergs/cm^2 led to complete decomposition of alanine and

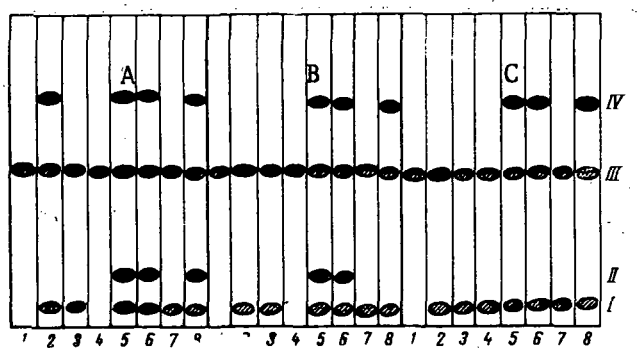


Figure 4. Chromatogram of Glycyleucine (0.01 M), Irradiated by Varying Doses in the Presence of Cations.

A, 2×10^9 ergs/cm^2; B, 4×10^9 ergs/cm^2; C, 6×10^9 ergs/cm^2; I, Glycine; II, Alanine; III, Glycyleucine; IV, Leucine; 1, Peptide, not irradiated; 2, Peptide irradiated in the presence of cations; 3, Zn^{2+}; 4, Al^{3+}; 5, Fe^{2+}; 6, Cu 6, Cu^{2+}; 7, Mn^{2+}; 8, Mixture.

the formation of glycine in the presence of Al^{3+} ions. During irradiation all of the peptides underwent deamination, which was sensitized by cations

$$\text{"Mixture"} > Cu^{2+} = Fe^{2+} > Mn^{2+} > Zn^{2+} = Al^{3+} > \text{peptide}.$$

Evidently the formation of ammonia in the glycylvaline and glycyleucine occurs due to decomposition of the NH_2-group and the —CO—NH—bond, but occurs in the gylcylalanine probably only as the result of the photochemical conversion of the NH_2-group of the glycine residue.

However, it is possible that during photolysis of the glycylalanine which decomposed under our conditions without the formation of free amino acids, nitrogen-containing substances were generated which did not develop as ninhydrins and which added their own contribution to the total magnitude of the deamination of glycylalanine (Leuschner et al., 1966).

The effect of the cations on the $\Phi_{(-m)}$ of the dipeptides gives the sequence:

$$Cu^{2+} > \text{"mixture"} > Fe^{2+} \geqslant \text{peptide} > Mn^{2+} \geqslant Al^{3+}.$$

We used phenylalanine (0.02 M) as an aromatic amino acid which was subjected to UV irradiation at λ = 254 nm in various gas media in the presence of polyphosphoric acid (PPA). The experiment showed that the photochemical changes of phenylalanine are accompanied by deamination of the NH_2--group, hydroxylation of the aromatic rings and formation of carbonyl substances, among which we determined formaldehyde and glyoxalic acid (Table).

Following photolysis spots appeared on the phenylalanine chromatogram, which were stained by the ninhydrin.

We identified tyrosine, phenylserine, alanine and serine (glycine), whose formation occurred in succession along with an increase in the irradiation dose.

The appearance of tyrosine and also of other hydroxyderivatives of phenyl- /320
alanine is confirmed by absorption spectra of eluates made from the unstained chromatographic strips. The maxima of absorption of the eluates is in the range 270-280 nm.

PHOTOLYSIS OF PHENYLALANINE IN AN ATMOSPHERE OF VARIOUS GASES.

$\Phi \cdot 10^{-3}$	In the absence of PFK					In the presence of PFK				
	Air	Ar	N_2	CO_2	Air (−78°)	Air	Ar	N_2	CO_2	Air (−78°)
(−m)	15,6	11,7	9,5	4,5	2,0	20,7	13,7	12,2	7,6	4,0
NH_3	4,2	3,4	1,7	0,5	0,9	5,5	4,3	1,9	1,0	1,1
—OH	5,0	2,4	3,9	1,4	0,25	6,8	3,4	5,0	2,6	0,45
C=O	4,6	2,9	3,4	1,2	0,75	8,8	3,9	4,2	2,3	1,0
CHO—COOH	13,6	9,2	11,0	8,3	—	20,5	13,6	13,6	12,7	—
H_2CO	1,6	0,7	0,5	0,8	—	2,5	1,5	0,9	1,4	—

Note: Commas indicate decimal points.

The solutions of phenylalanines frozen at -78° underwent chemical changes due to the ultraviolet effect. However, the Φ of the products being formed was significantly less than during irradiation of the liquid solutions of amino acids in air. During the chromatography we did not discover any substances which yielded a color with the ninhydrin. As a result of the photochemical conversions which occurred in the irradiated solutions of phenylalanine which were saturated with Ar, N_2 and CO_2, we determined the same products as we did during photolysis in air. However, their Φ appeared noticeably lower. From the table it is evident that progress of the photochemical reactions is most strongly inhibited in an atmosphere of CO_2.

The $\Phi_{(-m)}$ of the phenylalanine and the Φ_{NH_3} conforms to the sequence

$$\text{Air} > \text{Ar} > N_2 > CO_2,$$

but Φ_{OH}, $\Phi_{C=O}$ and $\Phi_{CHO-COOH}$ conform to the sequence

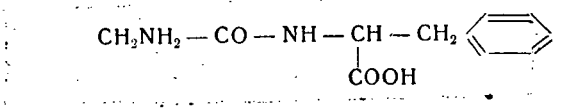
$$\text{Air} > N_2 > \text{Ar} > CO_2.$$

Thus, we see that hydroxylation and formation of carbonyl substances during photolysis is achieved in N_2 more actively than in Ar. However, the decomposition of phenylalanine and deformation occur with a greater Φ in Ar than in N_2.

The greatest Φ_{H_2CO} was found in the presence of air, and replacement of the latter by other gases reduced the Φ_{H_2CO} by half.

As can be seen in the table, the Φ of the glyoxalic acid was sufficiently high not only in air but also in the presence of other gases. During irradiation of an 0.02 M solution of alanine, a water-saturated solution of benzene (0.01 M) and a solution of alanine prepared in a solution of benzene in an atmosphere of air, we discovered that the glyoxalic acid was practically absent in the irradiated alanine ($\Phi = 0.2 \times 10^{-3}$) and was formed in a large quantity ($\Phi = 17.2 \times 10^{-3}$) in a solution of benzene. However, in a solution of alanine + + benzene the Φ of the glyoxalic acid was 9.8×10^{-3}.

These data lead us to suppose that the formation of glyoxalic acid during photolysis of phenylalanine occurs basically due to the photochemical conversion of the six-membered ring. During irradiation of the glycylphenylalanine in an atmosphere of air and Ar the peptide



underwent chemical conversion which in addition to hydroxylation in the formation of products of oxidative decomposition was accompanied by NH_3, CHO — —COOH, H_2CO of carbonyl substances, and also by the appearance of three amino acids — glycine, alanine and a new peptide — glycyltyrosine. When we increased the dose of irradiation (to 30×10^{18} ergs/cm^2) we detected traces of tyrosine, phenylalanine and a number of unidentified spots. Decomposition of the peptide occurred with a greater $\Phi_{(-m)}$, than for phenylalanine. The $\Phi_{(-m)}$ of the peptide in the air and in the argon was respectively 21.8×10^{-3} and 15.8×10^{-3}. /321

During irradiation of solutions of phenylalanine which contained polyphosphoric acid, which appeared as a macroergic compound, we discovered that the latter intensifies photolysis of an amino acid. For example, in an atmosphere of air the Φ of the C = 0-groups, OH and NH_3 increases respectively by 91 and 30%. The Φ of the decomposition of the amino acid itself increased by 33%. From the data in the table we concluded that the photosensitizing action of the PPA appears both during irradiation of the phenylalanine in an atmosphere of

Ar, N_2 and CO_2, and during deep cooling (-78°). The presence of PPA had a lesser effect on the photochemical conversion of the glycylphenylalanine. Thus, the $\Phi_{(-m)}$ of the peptide increased only by 15% during irradiation in air.

In our study of the ultraviolet action on solutions of aliphatic amino acids in the presence of oxides of metals we found that they intensify the photolytic spreading of the amino acids (Khenokh, Bogdanova, 1968). The effect of oxides on deamination of glycine, alanine and leucine can be presented in the form of the series:

$$CuO > Fe_2O_3 > TiO_2 > \text{amino acid} > ZnO.$$

This arrangement of oxides based on their activity in catalyzing the deamination of amino acids is also preserved during photolysis of frozen solutions (-78°). The action of oxides on the photochemical formation of glyoxalic acid in alanine and leucine is characterized by the following sequence: $CuO >$ amino acid $\geqslant SiO_2 > Fe_2O > ZnO$. This sequence is also correct during irradiation of amino acids in frozen solutions. However, deformation of dicarbohydrate fragments at -78° occurred more slowly than in solutions. The material produced enabled us to conclude that the accumulation of ordinary carbonyl compounds (substances which contain C = O-groups) during photolysis is sensitized by CuO, Cr_2O_3, SiO_2, and V_2O_5. Such oxides as TiO_2, Fe_2O_3 and beach sand had practically no effect (for example, in glycine) or slightly intensified (in leucine) the formation of carbonyl compounds. Suppression of photochemical reactions occurred in the presence of ZnO. It was shown chromatographically that irradiation of alanine (doses of $4.1\text{-}12.3 \times 10^9$ ergs/cm^2) in solutions and in the frozen state caused the formation of glycine. The addition of oxide did not change the chromatogram.

Under similar radiation conditions (doses of $8.2\text{-}24.3 \times 10^9$ ergs/cm^2) /322
photolytic conversions of valine led to the appearance of a number of new amino acids. Partial conversion of valine into thionine, alanine and glycine (which is also formed from valine in the absence of photosensitizers) occurred as a result of photolysis in the presence of V_2O_5, ZnO and Fe_2O_3. Full chemical decomposition of leucine is accompanied by appearance of glycine, but during the

addition of V_2O_5 we found alanine and two unidentified substances which contain amino groups.

The material presented in this article shows that various chemical reactions occur under the influence of ultraviolet in solutions of amino acids and peptides which are sensitized to radiation by various cations, oxides of metals and by polyphosphoric acids. This leads to breaking of the carbohydrate chain, hydroxylation of the aromatic ring and formation of products of oxidative destruction such as, for example, acids, carbonyl substances and ammonia due to the decomposition of the amino group. In addition to this new amino acids, in particular, those with a shortened chain of carbohydrate atoms, are formed. For example, leucine and norleucine split up under the effect of irradiation and in the presence of cations with the formation of glycine and alanine. Photolysis of the valine led to the appearance of glycine, and to threonine and alanine in the presence of oxides. The aromatic amino acid, phenylalanine, partially converted to aliphatic amino acids during irradiation, and also to new oxy-derivatives of phenylalanine. The longer the carbohydrate chain, the greater the Φ of the decomposition of the initial amino acid. In the series glycine → → alanine → norleucine (leucine) the $\Phi_{(-m)}$ was respectively 0.5×10^{-3}; 1×10^{-3} and 1.36×10^{-3}. For the indicated amino acids the photosensitizing effect, in particular, of oxides on an increase of the $\Phi_{(-m)}$ has the following sequence:

$$CuO > V_2O_5 > SiO_2 > Cr_2O_3 > \text{"beach sand."}$$

Certain distinctions are detected only for Fe_2O_3, TiO_2 and ZnO.

It has been shown in the photolysis of peptides that the greater the length of the dipeptide chain the greater its Φ of decomposition for an identical acyl radical. For glycylalanine, glycylvaline and glycyleucine the $\Phi_{(-m)}$ was respectively 0.25; 1.10 and 1.60×10^{-3}. Photolysis of the dipeptides did not necessarily lead to hydrolytic rupture of the —CO—NH— bond with liberation of free amino acids. Decarboxylation with preservation of the peptide bond may occur at this time, and so may the formation of free radicals which then

convert into the corresponding amines and amino acids (Leuschner et al., 1966). Cations catalyze the photochemical rupture of the –CO–NH bond in peptides, and with larger radiation doses they promote decomposition of new amino acids which /323 are formed.

From a comparison of the quantum yields of decomposition of different aliphatic amino acids and their peptides it can be hypothesized that the first synthesized peptide evidently consisted of amino acid with a shorter chain of carbohydrate atoms during the prebiological era of the existence of our planet (and, possibly, also during comparable conditions on other heavenly bodies). Such peptides could manifest a high degree of resistance to different physical factors and, in particular, to such a strong influence as ultraviolet radiation.

The results which we obtained show that changes which the abiogenically synthesized amino acids might be subjected to in the hydrosphere under the effect of ultraviolet light depend to a significant degree on the salt composition of the water and also on the ionizing radiation of soluble radioactive salts (Khenokh, Lapinskaya, 1955, 1958).

These data provide a basis for supposing that such ores as hematite (Fe_2O_3), rutile (TiO_2), zincite (ZnO), silica (SiO_2), Cr_2O_3 (in the form of chromite $FeOCr_2O_3$) and others (Vdovykin, 1967), in various geological periods of the prebiological existence of the Earth and possibly of other planets could have a catalytic effect on the photochemical conversions of amino acid, which somehow might have been concentrated (adsorbed) on the mineral surface.

It is essential to note that deep cooling (-78°) did not hinder the process of photolysis and the appearance of a catalytic activity of different cations of salts and minerals. The low temperature did slow down photolysis but did not halt it. The bioorganic compounds which arose under conditions of the primitive Earth (possibly also on other planets) could experience various chemical changes under the ultraviolet effect and not be dependent on temperature fluctuation.

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ACTION OF A SET OF EXTREMAL FACTORS ON RIBONUCLEASE

G. S. Komolova, Ye. V. Belikova and I. A. Yegorov

ABSTRACT: The action of a set of certain extremal factors on solutions of ribonuclease: ultraviolet light + x-ray radiation and ultraviolet light + repeated freezing and thawing were studied. The ultraviolet and x-ray radiation caused inactivation of the ribonuclease by means of similar mechanisms which were probably conditioned by chemical modification of tyrosine groups in the molecule. During the combined irradiation of ribonuclease by ultraviolet and x-rays we observed additive summation of their action.

Cryolysis causes denaturing changes in a molecule of ribonuclease which are different from those observed during photolysis. An irradiation of solutions of an enzyme increases its sensitivity to subsequent actions of freezing--thawing.

The task of investigating the biological action of a set of extremal /324
factors of outer space is an urgent one in connection with the conquest of space; among these factors ultraviolet and ionizing radiation and ultralow temperatures are quite important. The literature reveals only limited information on the combined action of these factors on biologically active substances. Nevertheless, such investigations are essential for the discovery of the mechanism of biological action of factors of outer space.

In this paper we have studied the combined action of ultraviolet and ionizing radiation and also the action of ultraviolet radiation and repeated freezing at ultralow temperatures on the enzyme ribonuclease.

Ribonuclease was selected as an object for investigation because of the sufficient study made of the structure of its molecule which facilitates the problem of explaining the nature of the denaturing changes of the enzyme caused by damaging factors.

We used a crystalline preparation of ribonuclease (2.7.1.15). We irradiated solutions of the enzyme (pH 6, phosphate buffer) with a concentration of 0.5 mg/ /mℓ. The concentration of the solutions which were subjected to repeated /325
freezing and thawing was 0.1 mg/mℓ.

These solutions were irradiated in a small flat-bottomed quartz cup. The volume of the irradiated liquid with a layer thickness of 2 mm was 1.5 mℓ. The temperature of the irradiated enzyme solution was maintained at zero degrees. We used an SBD-120A lamp as a source of ultraviolet light. The intensity of the ultraviolet light was in the range λ = 240-300 mμ, was measured by a UFI-65 dosimeter, and was 3.48 joules/m^2 sec during irradiation by only the ultraviolet, but it was 3.08 joules/m^2 sec during simultaneous combined irradiation by ultraviolet and x-ray radiation. The x-ray irradiation was conducted on an RUP-200 unit with a dose rate of 1,008 rads/min. During the combined irradiation of the ribonuclease solution the x-rays were directed at the object from above and the ultraviolet from below.

The repeated freezing-thawing of the 1.5 mℓ solutions of ribonuclease was conducted in test tubes made of calcined molybdenum glass. Samples were placed in liquid nitrogen for thirty minutes. Thawing took place in an air thermostat at a temperature of 20° for twenty-five to thirty minutes.

After this action the ribonuclease solutions were tested for enzyme activity by the Anfinsen method (Anfinsen et al., 1954).

We carried out spectral investigations of the enzyme solutions in order to determine the nature of the denaturing changes in the ribonuclease. Differential and ultraviolet spectra were recorded with the Unicum-700 automatic spectrophotometer. Optical density was measured on the SF-4 spectrophotometer.

Concentration of the enzyme solution for the spectral investigations was 0.5 mg/mℓ. In instances where the concentration of the starting solutions was 0.1 mg, they were evaporated on a rotary evaporator for the optical investigations until a final concentration of 0.5 mg/mℓ was attained.

Table 1 contains average results from the three determinations of the inactivation of ribonuclease by ultraviolet and x-ray radiation during their combined consecutive and simultaneous actions. We computed the theoretically expected result of inactivation during the joint action of the factors in the possibility of the additive effect according to previously taken dose curves of

inactivation of the enzymes for x-ray and ultraviolet radiation. Scatter in the results obtained in different tests was compensated by insertion of a correction computed on the basis of data for inactivation of an enzyme which were obtained in a specific test for ultraviolet and x-ray radiations made separately.

TABLE 1. EFFECT OF THE COMBINED ACTION OF ULTRAVIOLET AND X-RAY RADIATION ON SOLUTIONS OF RIBONUCLEASE.

Action	Doses of ultraviolet rays (in joules/m^2); x-rays (in rad)	Inactivation, %*	
		Expected in case of the additive effect	Obtained experimentally
Ultraviolet → x-ray	$0{,}5 \cdot 10^3 \rightarrow 100$	49	52
	$0{,}5 \cdot 10^3 \rightarrow 100$	43	43
	$1{,}0 \cdot 10^3 \rightarrow 100$	63	61
X-ray → ultraviolet	$100 \rightarrow 0{,}5 \cdot 10^3$	54	53
	$100 \rightarrow 0{,}5 \cdot 10^3$	58	60
	$100 \rightarrow 0{,}5 \cdot 10^3$	56	56
X-ray + ultraviolet	$100 + 1 \cdot 10^3$	54	56
	$100 + 1 \cdot 10^3$	57	56
	$100 + 1 \cdot 10^3$	59	61

*100% is understood to represent the activity of enzyme solutions before the action.
Note: Commas indicate decimal points.

From the results presented in Table 1 it is evident that the experimentally found values for inactivation of an enzyme during the combined action of ultraviolet and x-ray radiation on the solution of an enzyme are close to the theoretically calculated values of the solutions investigated with consideration of the possibility of additive action on the enzymes. /326

The differential ultraviolet spectra of irradiated solutions of ribonuclease, in comparison with non-irradiated solutions, show maxima in the range λ = 250-270 mμ at 275, 283 and 295 mμ during all three forms of action (ultraviolet, x-ray, ultraviolet + x-ray). The maximum at 295 mμ is the

sharpest. Clear minima occur at 278 and 287 mμ. Spectral changes increase with increase in enzyme inactivation in an equal degree for all forms of the actions studied. For an identical percentage of inactivation of ribonuclease in solution treated by x-ray, ultraviolet or by their combination in any quantitative ratio, the recorded differential spectra are superimposed on one another. The differential spectra for a solution of ribonuclease at 50% inactivation of an enzyme is presented in Figure 1.

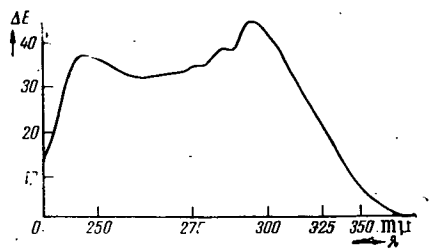

Figure 1. Differential Ultraviolet Spectrum of Irradiated Ribonuclease Against Native Ribonuclease (Inactivation is 50%).

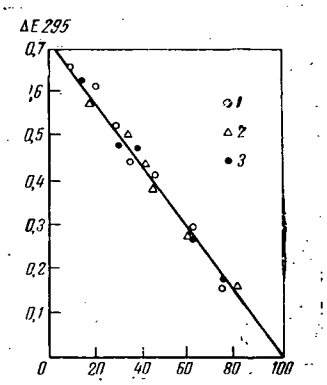

Figure 2. Relation of the Change in Optical Density of Solutions of Ribonuclease at 295 mμ to Percentage of Inactivation Caused by Ultraviolet (1), X-ray (2), Total Action of Ultraviolet and X-ray (1:1 by Percent of Inactivation (3).

Study of the absorption spectra of the irradiated solutions of ribonuclease shows an increase in optical density in the range of wavelengths 240-300 mμ. Figure 2 is a graph of the dependence of the increase of optical density of solutions at λ = 295 mμ to the degree of inactivation of the enzyme for x-ray, ultraviolet and their combined actions (1:1, by percent of inactivation). The graph reflects the direct relationship of change in optical density to percent of inactivation, while it is evident that the value ΔE for equal inactivation does not vary in margin of error for all /327 forms of the actions investigated.

We investigated repeated freezing--thawing and ribonuclease in another series of tests combined with ultraviolet radiation. Earlier we had shown that ribonuclease is inactivated during repeated freezing of its solution at a temperature of liquid nitrogen with subsequent slow thawing in air (Belikova, Komolova, Yegorov, 1968).

Table 2 presents data on the inactivation of ribonuclease during the action of repeated freezing-thawing on enzyme solutions irradiated by ultraviolet rays. Because inactivation of ribonuclease is directly related to the number of cycles of freezing-thawing, during the action of ultraviolet → freezing-thawing in the case of additive summation of effects produced by each factor, the values for inactivation which correspond to each factor separately must be totalled. However, as indicated from the data in the table, the experimentally obtained values for inactivation of an enzyme in samples which have been subjected to combined action of ultraviolet and cryolysis are noticeably higher than the theoretically expected values in the case of additive summation of the effects /328 caused by each factor. Obviously, latent changes which are caused by irradiation appear as damage in the process of freezing-thawing in a molecule of the enzyme.

TABLE 2. INACTIVATION (IN %) OF SOLUTIONS OF RIBONUCLEASE DURING THE COMBINED ACTION OF ULTRAVIOLET AND REPEATED FREEZING-THAWING (AVERAGE RESULTS OF THREE TESTS).

Ultra-violet	Freezing-thawing	Combined action: Ultraviolet → Freezing-Thawing	
		In case of additive summation of effects	Obtained experimentally
23	20	43	53
31	15	46	68
58	10	68	99

Freezing-thawing causes changes in the optical properties of solutions of ribonuclease which are at first manifested in an increase of optical density at 280 mμ. However, as can be seen from the data in Table 3, the ΔE (difference in optical density in control and test samples at λ = = 280 mμ) decreases as the percentage of inactivation of the enzymes as a result of cryolysis increases. During vigorous action, when the enzyme in the sample was completely inactivated, we also observed a reduction in optical density in the samples in comparison to the control samples.

TABLE 3. CHANGE IN OPTICAL DENSITY OF RIBONUCLEASE SOLUTIONS AS A RESULT OF REPEATED FREEZING-THAWING (AVERAGE RESULTS OF THREE TESTS)

Action	Inactivation, %	ΔE, in units of optical density, where λ = 280 mμ	Action	Inactivation, %	ΔE, in units of optical density, where λ = 280 mμ
Repeated freezing--thawing	15	0.024	Ultraviolet + + repeated freezing--thawing	20	0.039
	20	0.019		50	0.042
	30	0.014		80	0.020
	60	0.003			

From the data in Table 3 it follows that enzyme solutions subjected to /329
irradiation and repeated freezing (1:1, by percent of inactivation), have an optical density higher than solutions with the same percent of inactivation on an enzyme during cryolysis when λ = 280 mμ.

Figure 3 presents differential spectra for ribonuclease solutions which have been subjected to repeated freezing-thawing and the combined action of irradiation and freezing-thawing in comparison to control solutions (before the action).

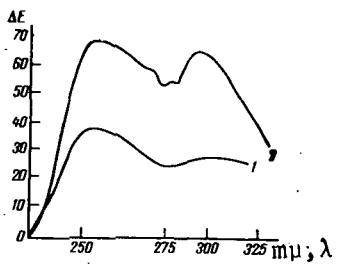

Figure 3. Differential Ultraviolet Spectra of Ribonuclease as Against a Native Solution. 1, During repeated freezing the enzyme solution (inactivation is 20%); 2, During consecutive action of ultraviolet and repeated freezing-thawing of the enzyme solution (inactivation is 83%).

Noticeable maxima are observed at λ = 250 and 280 mμ on the differential spectrum obtained for cryolysis. At longer wavelengths we observed a significant increase in ΔE due to scattering of light which was conditioned by the formation of aggregates of molecules as a result of coagulation. Optically the properties of the solutions which were subjected to cryolysis were conditioned on the one hand by an increase in scattering of light and on the other by

precipitation of a coagulant into the residue which is explained by the reduction in optical density when λ = 280 mμ on an occasion when there was a large number of freezing-thawing cycles. The maximum observed in the differential spectrum when λ = 250 mμ was due to the appearance of a new product as a result of cryolysis.

The spectral properties of ribonuclease during combined action basically preserve the features characteristic for photolysis as is evident from the differential spectra for the solution of ribonuclease which was subjected to irradiation by ultraviolet before cryolysis. This was also manifested in the higher level for the ΔE values found for solutions of an enzyme treated by the combined action. However, cryolysis also has a definite effect on the optical properties of a solution of an enzyme during the combined action which is manifested in increased scattering of light and a significant increase in the maximum at $\lambda \leqslant 250$ mμ. The product which determined its introduction is formed during photolysis although its role in the mechanism of inactivation of the enzyme during these forms of action is obviously varied.

The maximum values observed in the differential spectra of ribonuclease which was irradiated by x-ray and ultraviolet radiation, and which are displaced in comparison with the maximum for tyrosine, undoubtedly confirm the /330
chemical modifications of tyrosine. It is known that tyrosine may oxidize to phloxyphenylalanine (DOFO) under the effect of ultraviolet (Roshchupkin, 1966). Formation of such a product is also probable during the action of x-ray radiation (Collinson, Dainton, Holmes, 1959). It is known that phenyl groups participate in maintenance of native conformation of ribonuclease so that their oxidation could cause inactivation of a molecule of an enzyme (Cha, Scheraga, 1960). Formation of tyrosine oxidation products causes the observed growth of optical density for the irradiated solutions of ribonuclease in the wavelength range 240-300 μ^2. It is possible to assume that the inactivation of ribonuclease caused by ultraviolet and x-ray is determined by the break of the hydrogen bonds between the tyrosine groups which enter directly into the catalytic center. Identical quantitative ratios between the degree of inactivation and spectral changes of the enzyme solution for all of the actions

studied confirm the identity in the mechanism of reaction. For the combined action of x-ray and ultraviolet the accuracy of the equation: effect of ultraviolet + effect of x-ray = effect of the combination (ultraviolet + x-ray) must result from identity in their action on the enzymes. This is confirmed experimentally by an experiment in determining the ribonuclease activity and in studying spectral characteristics of enzyme solutions. The enzyme inactivation mechanisms during cryolysis and photolysis are not identical, as indicated by various changes of optical properties of enzyme solutions during the action of one or the other. Therefore we assume that during photolysis of the ribonuclease, in addition to the basic processes which lead the enzyme to inactivation, reactions are created which determine modification of certain groups in a molecule which do not lead to its inactivation alone and without additional actions. However, under specific conditions the product formed can react with the neighboring polypeptide chain and form a cross-link with it which is a direct cause for the denaturizing changes in the molecule which are accompanied by inactivation (Vladimirov, 1965). We suppose that the increase of inactivation of the irradiated enzymes due to freezing-thawing which we observed was conditioned by the formation of cross-links between neighboring polypeptide chains by means of the active product formed in the process of irradiation. Realization of the ultraviolet-induced changes of different groups of the enzyme into configuration to cause damage to the molecule by means of formation of cross-links probably occurs as a result of binding of the polypeptide chains during the process of freezing at ultralow temperatures.

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EFFECT OF A GAS ENVIRONMENT ON CATALASE CRYOLYSIS

G. S. Komolova

ABSTRACT: Catalase is inactivated during repeated freezing and thawing of its solutions. The effect depends on the temperature of freezing and the gas atmosphere. In an atmosphere of argon there is no inactivation of catalase during freezing and thawing. Other gases tested for the degree of their effect on cryolysis in order of increasing effect on inactivation of the enzymes are: N_2, He, O_2, H_2.

In a gas environment consisting of oxygen and nitrogen we observed additive summation of the effects on cryolysis which were produced by each gas separately. In addition, investigation of the effect of an atmosphere consisting of hydrogen and argon (1:1, by volume) and cryolysis of an enzyme revealed a significant increase of inactivation in comparison to the expected motivation for the case of additive summation of effects produced by each gas separately.

Results obtained confirm the necessity of considering the composition of a gas atmosphere in evaluating the damaging action at low temperatures on biologically important substances.

Investigations of the biological action of low temperatures at all levels /331
of biological organization are becoming increasingly important in connection with spaceflights.

It is known that certain isolated enzymes in solution lose their activity under the action of repeated freezing and thawing (Kiermeier, 1948; Precht et al., 1955; Schikama, Yamasaki, 1961; Hanafusa, Naofumi, 1967). Freezing-thawing conditions (temperature of freezing, rate of thawing) and nature of the medium (pH of the solution, presence of certain substances) have an effect on the degree of inactivation of enzymes. It is assumed that cryolysis of biologically active substances under conditions on other planets and in outer space will differ from those on Earth. Above all, the significantly varying nature of the gas atmosphere on various planets merits attention. However, the problem of the effect of the gas environment on the biological action of low temperatures has not received the necessary attention in the literature on the subject.

In this paper we have attempted to investigate the effect of the nature of the gas environment on the cryolysis of an enzyme (catalase) which is quite resistant to the action of freezing and thawing.

We tested solutions of crystalline catalase (1.11.1.6) in a concentration of 1.6×10^{-3} mg/mℓ (pH = 7.0, phosphate buffer).

These solutions were prepared in doubly distilled water which was previously degassed by means of boiling and freezing out. For the freezing and thawing period we placed 1 mℓ of enzyme solution in special 15 mℓ quartz vessels /332 to which a bubble cap, which extended into a capillary tube in its upper portion, was fitted by means of ground glass joints. The tested gases were fed through the capillary tube into the vessel until a predetermined pressure had been reached in the space above the solution, and then the capillary tube was sealed. We bubbled the gases through the enzyme solution at 0°. Pumping the air out of the vessel before feeding the gas into it was carried out at a pressure of 10^{-2} atm. Pressure of the tested gases above the solution was 1 atm.

Before the final filling of the vessel with the gas the procedure of decanting and replacing some of it was repeated several times. The gases (nitrogen, hydrogen, argon and helium) were purged of oxygen impurities by passing them through a solution of pyrogallic acid.

Negative temperatures were reached (from -10 to -40°) in an I-6 ultrathermostat and lower temperatures were reached (from -40 to -180P) in an East German-made cryostat. Temperature in these devices was maintained with an accuracy of ±1°.

The solutions were kept at the test temperatures for thirty minutes during the freezing process. Thawing of the solution in water at a temperature of +25° proceeded for 2 to 2.5 minutes. Final temperature of the enzyme solution after thawing did not exceed +4°.

The control samples were maintained at 0° during the freezing and thawing of the test samples.

We judged the results of the action of freezing and thawing on the enzyme solutions by the change in enzyme activity which we determined by the

spectrophotometric method (Beers, Sizer, 1952). Results were expressed in percentages of the value of the amount of enzyme activity in the control solution. Several repetitions of the freezing and thawing of the catalase solutions caused inactivation of the enzymes. The effect depended on the number of freezing and thawing cycles. The average results of the four tests follow.

Number of cycles	1	4	10	15
Analysis of activity, %.....	0	4 ± 2	21 ± 6	32 ± 3

In Figure 1 curve A shows the relationship of inactivation of the catalase to freezing temperature during freezing and thawing of the enzyme solution in an atmosphere of air. The greatest inactivation on the curve is observed at the temperature interval from -30 to -100°, while no relationship of the effect of freezing and thawing to temperature is observed in this temperature interval. However, a further reduction in temperature is accompanied by a corresponding reduction of effect.

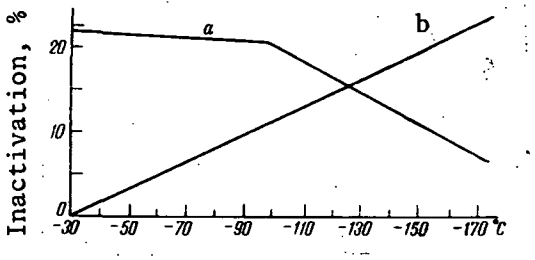

Figure 1. Relationship of Catalase Inactivation to Temperature of Freezing of Enzyme Solutions in an Atmosphere of Air (a) and Hydrogen (b).

Degassing of the solution as well as replacement of the air by oxygen, nitrogen or helium does not influence the effect during freezing of the enzyme solutions in the temperature interval from -30° to -100°. /333 At these temperatures only argon and hydrogen gave results different from those obtained with air. At the given temperature interval inactivation of the enzyme was practically absent in an atmosphere of argon and was reduced in comparison to air in an atmosphere of hydrogen. One should note the fact that the nature of the temperature relationship of catalase inactivation in an atmosphere of hydrogen is somewhat different than in atmospheres of the other tested gases. As can be seen in

Figure 1, inactivation is directly related to the freezing point in an atmosphere of H_2.

At ultralow temperatures (below -100°), the nature of the gas atmosphere in inactivation of the enzymes during freezing and thawing has a significant effect for all of the gases. As can be seen from Figure 2, graded according to increasing intensification of the freezing and thawing effect on catalase, the tested gases are arranged in the following order: Ar, N_2, He, O_2, H_2.

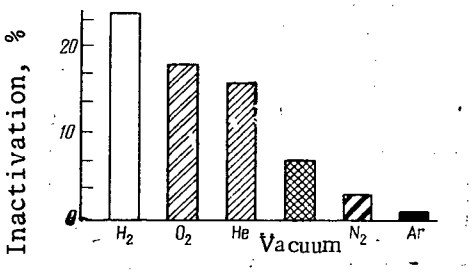

Figure 2. Inactivation of Catalase During Repeated Freezing and Thawing of Enzyme Solutions in Atmospheres of Various Gases (Freezing Point was -180°).

Table 2 presents data on the effect of a mixture of certain gases on catalase cryolysis. From the data presented it follows that summation of the effects which take place in an atmosphere of each gas separately is observed during freezing and thawing of catalase solutions in an atmosphere of mixed nitrogen and oxygen (in a ratio of 1:1, but according to pressure). Other results were obtained by mixing argon and hydrogen (in a ratio of 1:1 by pressure). Inactivation of catalase during freezing and thawing of its solutions in an atmosphere of mixed argon and hydrogen was higher than the expected value in the case of additive summation of effects induced by each gas separately. This increase in effect, as can be seen from the data presented in the table, is especially significant in the case of the freezing of enzyme solutions at a temperature of -70°. /33

The many factors involving the mechanism of pyrolysis require that a different approach be taken in its investigation to explain the optimum conditions for the appearance of the action of the different factors. From curve a of Figure 1 it follows that cryolysis of catalase in air in the temperature interval (-30 to -100°) does not depend on the freezing point.

For the given temperature interval damage to the enzyme is obviously determined by the mechanical factor, whose nature is related to the development of microscopic cracks in the ice. It is known that atmospheric gases remain soluble in water at 0°, and that the gas bubbles formed during freezing can get into the microscopic cracks formed in the ice (Smit, 1962). However, as our test results showed, the action of the given factor in the presence of the majority of the tested gases was identical. Exceptions were argon and hydrogen, in whose atmospheres cryolysis was suppressed in the temperature range (-30 to -100°). At freezing points below -100° the action of freezing and thawing on catalase depends on the freezing point. Action of a factor which appears under these conditions weakens as the rate of the liquid phase-solid phase transition increases.

Evidently this factor manifests its action in the interstitial phase which is shortened as the rate of freezing increases. According to concepts of mechanochemistry (Baramboym, 1961; Berlin, 1963) ruptures of the covalent bonds in the macromolecules occur during the growth of crystals in the interstitial phase, which may allow organic radicals to be generated which are capable of causing chain reactions under specific conditions.

Protein molecules which are drawn into the chain reaction undergo changes which lead to their denaturing.

EFFECT OF THE MIXTURE OF GASES ON CRYOLYSIS OF CATALASE. (AVERAGE RESULTS OF SIX TESTS, INACTIVATION, %).

	$N_2 + O_2$ (1:1)				$Ar + H_2$ (1:1)			
Temperature, °C	N_2	O_2	Expected in the case of summation of effects	Experimentally obtained	Ar	H_2	Expected in the case of summation of effects	Experimentally obtained
—70	26±1	25±0,5	25,5±0,7	23±1,3	1±0,6	7±2	4±1,3	35±1,7
—180	3±0,8	18±0,3	10,5±0,5	9±1,2	9,5±0,5	24±1,9	12,2±1,2	20±1,3

Note: Commas indicate decimal points.

Peroxide radicals with a high reactivity are formed in the presence of oxygen:

/335

$$Rm \xrightarrow{\downarrow} Rm^{\cdot} + Rn^{\cdot}$$
$$Rm^{\cdot} + Rn^{\cdot} \xrightarrow{O_2} RmOO^{\cdot} + RnOO^{\cdot}.$$

The independence of catalase cryolysis of the presence of oxygen in the medium in the temperature range (-30 to -100°) confirms the fact that the inactivation mechanism, which is related to the development of radical reactions, is excluded under these conditions. At the same time a noticeable tendency to an increase in enzyme inactivation is observed at temperatures below -100° during freezing and thawing with an increase of partial pressure in a gas medium of oxygen.

Obviously, under conditions of ultralow temperatures, a mechanical factor determines the origin of reactions which must be assigned a definite role in catalase inactivation.

The fact that the nature of the temperature relationship of catalase inactivation during freezing, identical for the majority of gases tested, differed for hydrogen, also confirms the action of different factors under conditions of freezing and thawing of enzyme solutions in various gas atmospheres. We cannot yet identify all the factors responsible for cryolysis and discover the cause of their varying behavior in various gas atmospheres, but our data fully confirm the necessity of considering the nature of the gas environment in investigating the cryolysis mechanism of biologically active substances.

Tests with mixtures of gases show that summation of the effects produced by each gas separately is observed when mixing gases which form the basis of the Earth's atmosphere (N_2 and O_2). However, the observed phenomenon is not true of all gases. Mixing hydrogen and argon revealed a significant increase of the effect in comparison to the expected effect in the case of summation of effects induced by each gas separately. Such behavior of gas mixtures should also be considered in evaluating the biological action of low and ultralow temperatures under conditions of gas atmospheres which are different from those on Earth.

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